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VARIETAL DIFFERENCES IN BARLEYS AND MALTS

VIII. CORRELATIONS BETWEEN ENZYMATIC ACTIVITIES AND MALT EXTRACT, MALTING LOSS, AND STEEPING TIME[1]

BY HENRY R. SALLANS[2] AND J. ANSEL ANDERSON[3]

Abstract

Significant negative inter-varietal correlation coefficients between steeping time and barley saccharifying (−0.706), malt saccharifying (−0.814), starch-liquefying (−0.954), autolytic diastatic (−0.937), and proteolytic (−0.822) activities are shown to be dependent on the same fundamental barley properties as similar positive coefficients between these activities and salt-soluble barley nitrogen. Barley and malt saccharifying and proteolytic activities are equally associated with both salt-soluble nitrogen and steeping-time, but starch-liquefying and autolytic diastatic activities are more closely associated with the latter property. Significant inter-varietal correlation coefficients were obtained between liquefying, autolytic, and proteolytic activities and malt extract and malting loss. Partial correlation studies indicate that only the simple coefficient between malting loss and proteolytic activity (0.915) represents a real relation independent of salt-soluble nitrogen and steeping time.

With the exception of autolytic diastatic activity, highly significant intra-varietal correlation coefficients were obtained between malt extract and enzymatic activities. Partial coefficients, independent of total nitrogen, show that proteolytic, liquefying, and autolytic activities are associated with malt extract. Simple intra-varietal correlations of malting loss with barley saccharifying (0.671), malt saccharifying (0.702), and proteolytic (0.701) activities reflect mainly the effects of total nitrogen. Enzymatic activities are negatively correlated with steeping time within as well as between varieties, and partial correlation coefficients suggest a real relation between liquefying activity and steeping time.

Other papers in this series dealt with a number of barley, malt, and malting properties determined on the same set of 144 samples. The data obtained have made it possible to investigate the relations between a comparatively large number of pairs of properties. While all of these relations are not of equal importance or interest many of them are worth recording.

The development of enzymes is fundamental in the malting process and it seemed of value to study the interrelations of enzymatic activities with certain malt and malting properties. The present paper contains a discussion of the relations between barley saccharifying activity, saccharifying, starch-liquefying, autolytic diastatic, and proteolytic activities of malt on the one hand and malt extract, malting loss, and steeping time on the other.

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Data

The experimental data were obtained by analysis of 144 samples of barley and of the malts made from them. The samples represent 12 varieties grown at 12 widely separated experimental stations in Canada (1). Malting methods and summaries of the data on malt extract, malting loss, and steeping time were given in Part IV (4). Data on saccharifying activities of the barleys and malts were discussed in Part II (5) and Part III (2), those on proteolytic activity in Part VI (3), and those on starch-liquefying and autolytic diastatic activities in Part VII (6).

Correlation Studies

It was shown in previous papers (2, 3, 6) that significant inter-varietal relations existed between non-protein, salt-soluble protein, and total salt-soluble barley nitrogen and enzymatic activities, but that correlations of these activities with the other nitrogen fractions were not significant. In the case of intra-varietal relations the correlation coefficients between the enzymatic activities and total nitrogen, insoluble nitrogen, and alcohol-soluble nitrogen are highly significant, while those between these activities and the non-protein, salt-soluble protein, and total salt-soluble nitrogen represent a lesser degree of association. Thus, it appears that inter- and intra-varietal relations between enzymatic activities and other fundamental barley and malt properties are different in nature. For these reasons the inter- and intra-varietal relations are discussed in separate sections.

Inter-varietal Relations

The most interesting feature of the present study is that highly significant negative inter-varietal correlation coefficients were obtained between enzymatic activities and the time required to steep the barleys to a moisture content of 46%. In Part VII (6) it was shown that similar positive coefficients were obtained between the enzymatic activities and salt-soluble barley nitrogen. These simple correlation coefficients together with the multiple coefficients between the activities and salt-soluble nitrogen and steeping time are given in Table I. It appears that varieties that are high in salt-soluble nitrogen tend to yield malts of high enzymatic activity and those that require a long steeping time tend to yield malts of low activity.

The correlation coefficients in Table I were determined from variety means, and do not give information concerning the associations at any particular station. To determine whether they represent fundamental relations between enzymatic activities and steeping time, a more intensive study was undertaken. Regressions of the activities on steeping time were determined by stations and tested for homogeneity. From the results of these analyses, shown in Table II, it appears that the regressions do not differ significantly from station to station. Thus unit change in steeping time is accompanied by the same change in any given activity at all stations. This indicates that real relations exist between steeping time and the enzymatic activities.

Mean square residuals from the individual regressions of the activities on steeping time were tested against similar values for the regression on salt-soluble nitrogen (cf. 6, Table VI). It was found that the residual variances of barley saccharifying, malt saccharifying, and proteolytic activities did not differ significantly; but regressions of starch-liquefying and autolytic diastatic activities on steeping time accounted for significantly greater portions of the total variances of these activities than similar regressions on salt-soluble nitrogen. It may therefore be concluded that barley saccharifying, malt saccharifying, and proteolytic activities are equally associated with both salt-soluble nitrogen and steeping time; but that starch-liquefying and autolytic diastatic activities are more closely associated with steeping time than with salt-soluble nitrogen.

The multiple correlation coefficients between the enzymatic activities and salt-soluble nitrogen and steeping time were determined and are shown in the last column of Table I. Tests of residual variance (cf. 4, Table IX) showed

TABLE I

INTER-VARIETAL CORRELATION COEFFICIENTS AMONG ENZYMATIC ACTIVITIES (x) SALT-SOLUBLE BARLEY NITROGEN (n) AND STEEPING TIME (s)

x = enzymatic activity	Correlation coefficient		
	Simple r_{xn}	Simple r_{xs}	Multiple $R_{x.ns}$
Barley saccharifying	.739**	−.706**	.776**
Malt saccharifying	.727**	−.814**	.835**
Malt liquefying	.727**	−.954**	.955**
Autolytic diastatic	.750**	−.937**	.941**
Proteolytic	.871**	−.822**	.909**

NOTE: *In this and the following tables, ** indicates that the 1% level, and * that the 5% level of significance has been attained.*

that these multiple coefficients would not account for significantly greater portions of the variance than the higher of the two simple correlation coefficients. These considerations together with the fact that a significant simple correlation (−0.740**) exists between salt-soluble nitrogen and steeping time, indicate that the associations of steeping time and salt-soluble nitrogen with enzymatic activities are due to the same fundamental causes. It is difficult to interpret these data in terms of the physical and chemical properties of the barley. However, one appears forced to the conclusion that the soluble nitrogen fractions of the barley or some related factors influence the rate of absorption of water in the steep. Furthermore, it seems reasonable to assume that the relations between enzymatic activities and steeping time may be due to physical and chemical properties of the barleys that influence the rate of diffusion within the kernels.

It is evident from Table II that regressions of enzymatic activities on steeping time do not account for sufficiently high percentages of the variances of the activities to permit their prediction from steeping time. But, since both salt-soluble nitrogen and steeping time are related to all the activities, it is possible that these properties might be used in conjunction with a measure of potential extractives for the prediction of extract.

TABLE II

TEST OF HOMOGENEITY OF INTER-VARIETAL REGRESSION OF ENZYMATIC ACTIVITIES ON STEEPING TIME BY ANALYSIS OF RESIDUAL VARIANCE

Variance due to	Degrees of freedom	Mean square				
		Barley saccharifying	Malt saccharifying	Starch liquefying	Autolytic diastatic	Autolytic proteolytic
Differences among station regression coefficients	11	553.849	195.413	3,132.3	5,351.36	2,739.85
Deviations from individual station regressions	120	805.402	329.981	8,274.9	6,209.92	1,805.51
Percentage of intra-station variance accounted for by individual regressions		30.0	40.2	63.3	46.5	40.3

In Part VII (6) it was shown that the associations between enzymatic activities were due mainly to their common association with salt-soluble nitrogen. Since steeping time accounts for the same portions of the variance of the activities plus a greater portion in the case of liquefying and autolytic activities, partial correlation coefficients between the enzymatic activities, independent of both salt-soluble nitrogen and steeping time, were determined. These are shown in Table III. Only two are significant, namely, barley saccharifying—malt saccharifying and liquefying—autolytic.

It was demonstrated in Part VII (6) that determinations of barley and malt saccharifying activities were measures of β-amylase, and determinations of starch-liquefying and autolytic activities were measures of α-amylase. It is

TABLE III

INTER-VARIETAL PARTIAL CORRELATION COEFFICIENTS, BETWEEN ENZYMATIC ACTIVITIES, INDEPENDENT OF BOTH SALT-SOLUBLE NITROGEN AND STEEPING TIME

Enzymatic activity	Malt activities			
	Saccharifying	Liquefying	Autolytic diastatic	Proteolytic
Barley saccharifying	.785**	.351	.289	−.276
Malt saccharifying	–	.209	.417	−.344
Malt liquefying	–	–	.870**	.013
Malt autolytic diastatic	–	–	–	.028

therefore not surprising that the partial correlation studies indicate fundamental relations between these properties. The remaining coefficients all fall well below the 5% level of significance, and it appears that no direct association exists between these activities apart from their common association with certain barley properties. Salt-soluble nitrogen and steeping time reflect these physical and chemical properties, and it seems logical to use them as measures of potential enzymatic activity in studying the prediction of extract.

Inter-varietal correlation coefficients between enzymatic activities and malt extract and malting loss are shown in Table IV.

TABLE IV

INTER-VARIETAL SIMPLE CORRELATION COEFFICIENTS BETWEEN ENZYMATIC ACTIVITIES AND MALT PROPERTIES, AND PARTIAL COEFFICIENTS INDEPENDENT OF BOTH SALT-SOLUBLE NITROGEN AND STEEPING TIME

Enzymatic activity	Correlation coefficient			
	Malt extract		Malting loss	
	Simple	Partial	Simple	Partial
Barley saccharifying	.288	−.266	.436	−.642*
Malt saccharifying	.325	−.357	.465	−.675*
Malt liquefying	.592*	.136	.695*	.060
Malt autolytic diastatic	.653*	.362	.700*	−.005
Malt proteolytic	.644*	.448	.915**	.689*
Residual degrees of freedom	10	8	10	8

The simple coefficients in the first column indicate that associations exist between malt extract and liquefying, autolytic, and proteolytic activities; so that varieties which tend to be high in these activities tend to be high in extract also. The corresponding partial coefficients independent of salt-soluble nitrogen and steeping time are not significant and suggest that the physical and chemical factors responsible for the inter-varietal relations between the enzymatic activities also affect the extract yield. This hypothesis is supported by the fact that the simple correlation coefficient between malt extract and steeping time (−0.590*) was significant.

The simple correlation coefficients between enzymatic activities and malting loss are very similar to but higher than those for extract. The simple coefficient for proteolytic activity (0.915), computed from variety means, although quite high, does not, however, represent a particularly close relation in individual cases, for the regression of proteolytic activity on malting loss by stations accounted for only 46.6% of the total intra-station variance of proteolytic activity. However, the significant partial correlation independent of salt-soluble nitrogen and steeping time does suggest that proteolytic enzymes are an important factor controlling malting loss. It would appear that varieties well supplied with proteolytic enzymes grow vigorously, with

the result that malting loss tends to be high. The partial coefficients for liquefying and autolytic activities show that these have little effect on malting loss when the effects of salt-soluble nitrogen and steeping time are removed.

An apparent anomaly occurs in the case of partial coefficients, independent of salt-soluble nitrogen and steeping time, between malting loss and saccharifying activities. It is difficult to understand why these should be significant and negative. However, when the data were plotted, it was observed that for unit decrease in steeping time the saccharifying activities did not increase as much as would be expected from the increase in malting loss. This effect was particularly marked with the varieties Charlottetown 80, Hannchen, Victory, Regal, and Velvet, and is sufficient to account for the negative partial coefficients. Since the simple coefficients for saccharifying activities and malting loss are not significant and the partials are negative, it must be concluded that these activities are not related to malting loss. This was to be expected, because it was shown in Part VII (6) that the liquefying or α-amylase activity was the most important factor governing autolysis in the malts and that saccharifying or β-amylase activity had no significant effect on this reaction.

Intra-varietal Relations

The intra-varietal relations between enzymatic activities and malting properties are shown in Table V. In view of the significant correlation of enzymatic activities with total nitrogen (6) and with certain of these malting properties, partial coefficients were determined to show whether relations exist independent of a common association with total nitrogen.

TABLE V

INTRA-VARIETAL SIMPLE CORRELATION COEFFICIENTS, AND PARTIAL COEFFICIENTS INDEPENDENT OF BARLEY NITROGEN, BETWEEN ENZYMATIC ACTIVITIES AND MALT PROPERTIES

Enzymatic activity	Correlation coefficient					
	Malt extract		Malting loss		Steeping time	
	Simple	Partial	Simple	Partial	Simple	Partial
Barley saccharifying	−.918**	.248	.671*	−.039	−.626*	−.503
Malt saccharifying	−.931**	−.143	.702*	.178	−.661*	−.228
Malt liquefying	−.728**	−.547	.281	−.282	−.881**	−.801**
Malt autolytic diastatic	.084	−.619*	.189	.542	−.269	−.593
Malt proteolytic	−.949**	−.874**	.701*	.290	−.704*	−.400
Residual degrees of freedom	10	9	10	9	10	9

Simple correlation coefficients between the enzymatic activities and malt extract indicate that within varieties, with the exception of autolytic activity, environmental conditions which increase enzymatic activities tend to decrease extract. The partial coefficients for proteolytic and autolytic diastatic activities are significant and that for liquefying sufficiently high to suggest that

these relations between extract and the activities are not entirely explained by association of both factors with total nitrogen. However, it appears that in general an increase in total nitrogen within varieties is accompanied by an increase in enzymatic activity and a decrease in extract yield.

The relation between proteolytic activity and extract is particularly interesting, since the high partial coefficient suggests that quite a close association exists between extract and proteolytic activity apart from a common association with total nitrogen. The multiple correlation coefficient for extract and proteolytic activity and total nitrogen proved to be 0.990, which is significantly higher than either of the simple coefficients. It appears that the relation between nitrogen and extract, which is negative, reflects a positive relation between the carbohydrates and extract; and the addition of proteolytic activity in the relation gives the added effect of the soluble nitrogen on malt extract.

There seems to be some intra-varietal association between malting loss and saccharifying and proteolytic activities. The partials, however, suggest that the simple correlation coefficients reflect mainly the correlations between each variable and total nitrogen.

Steeping time and enzymatic activities are negatively associated within as well as between varieties. The partial correlation coefficients show also that starch-liquefying activity is highly correlated with steeping time independent of the effect of total nitrogen. The inter- and intra-varietal associations between liquefying activity and steeping time appear to be so close that one is forced to conclude that some causal relation must exist between them. It appears that the longer steeping time required to reach a given moisture content reflects a greater resistance to absorption of water and possibly also to movement of water within the kernel. This might well influence both the rate of activation of enzymes and their rate of reaction with substrates.

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QUALITATIVE STUDIES OF SOIL MICRO-ORGANISMS

III. INFLUENCE OF PLANT GROWTH ON THE CHARACTER OF THE BACTERIAL FLORA[1]

By A. G. Lochhead[2]

Abstract

Comparative studies of the relative incidence of bacterial types occurring in the rhizosphere of different plants and in control soils indicated that the qualitative nature of the soil microflora is markedly influenced by the growing plant. In the rhizosphere Gram-negative rods are proportionately increased while Gram-positive rods, coccoid rods, and spore-forming types are relatively less abundant.

The majority of bacteria isolated from soil by non-selective plating are forms included in the family Proactinomycetaceae (Jensen's classification). Of these by far the largest group consists of members of the genus *Corynebacterium*. In the rhizosphere proactinomycetes as a whole are relatively less abundant, with the *Corynebacterium* (non-motile) group likewise depressed. However, closely related motile forms classed as *Mycoplana* are preferentially stimulated.

In the rhizosphere the bacteria show definitely greater physiological activity than in soil distant from the plant. Not only is there a notably greater proportion of motile forms, and a pronounced increase in the incidence of chromogenic types, but also a higher incidence of liquefying bacteria and of those able to affect glucose.

A comparison of the rhizosphere of certain plant varieties resistant and susceptible respectively to soil-borne disease showed differences of a qualitative nature in the bacterial flora suggestive of a greater "rhizosphere effect" in the case of the susceptible varieties studied. Results point to the possibility that resistance may be associated with a selective action of root excretions on the saprophytic soil microflora.

Introduction

In a previous paper of this series (16), following a general introduction (9), a report was made of investigations carried out on a non-selective basis of the qualitative nature and relative incidence of the bacterial types occurring in soils differing in fertility. On the basis of morphology in soil extract semi-solid medium, in which the organisms were considered to correspond more closely to their form in soil than when cultivated on more "synthetic" substrates, the bacteria were classified into eight groups. In spite of unequal productivity the soils showed no outstanding differences in the relative abundance of the various morphological groups, while grouping on the basis of physiological activity likewise showed a surprising degree of uniformity in the soils studied. A brief summary of the characters of the organisms found is given in Table I, recalculated from previous data for comparison with findings as to the effect of plant growth presented below.

The results suggested that the character of the *autochthonous* (indigenous) soil microflora is relatively uniform in soil of definite type, and comparatively

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little affected by normal field application of fertilizers resulting in greatly altered crop-producing capacity. The predominant soil bacteria appeared relatively inactive in single culture, with considerable divergence in biochemical action shown by apparently closely related forms. The findings suggested that the bacterial flora of soil is unstable physiologically, with considerable adaptability, and that the functions of the different types are exercised most fully only under conditions of association.

The object of the present investigation was to study the effect of the growing plant upon the nature of the soil bacteria, and compare the characters of bacteria in the rhizosphere with those of organisms away from the zone of influence of the plant.

The Rhizosphere—Qualitative Aspects

Some 35 years ago Hiltner (1) observed that certain micro-organisms showed increased activity close to the roots of cultivated plants and called this zone influenced by root excretions the "rhizosphere". Since then various other investigators have confirmed his findings, and shown that soil in the vicinity of the roots supports usually noticeably higher numbers of micro-organisms than soil more distant from the plant. Purely quantitative considerations, however, need not concern us here.

Our information as to the effect of plant growth on the qualitative nature of soil bacteria is meagre, and confined largely to data on the numbers of organisms appearing on selective media chosen to favour various groups. Thus Starkey (13, 14) found that the development of higher plants exerted greater influences upon some groups of organisms than upon others. Whereas the proportionate increases in nitrogen-fixing organisms, actinomycetes, and filamentous fungi were slight, organisms of the *B. radiobacter* group were preferentially stimulated in the rhizosphere. It was found, however, that different plants exerted different degrees of influence upon soil micro-organisms.

Krassilnikov (6) reported that *Azotobacter* was unable to grow in the rhizosphere of wheat and was severely repressed in that of maize, and attributes this to the toxic effect of root secretions. Similarly *B. mycoides*, *B. megatherium*, and yeasts of the type of *S. cerevisiae* were unable to grow in the rhizosphere of the same plants. On the other hand, small non-sporing bacteria of the types of *B. denitrificans* and *B. fluorescens* were found to multiply intensively in the rhizosphere, presumably under the stimulation of organic root excretions.

Evidence of a selective action exerted by the root system on soil bacteria was obtained by Krassilnikov, Kriss, and Litvinov (7), who reported striking increases in cellulose-decomposing organisms and of small non-spore-forming rods growing poorly under laboratory conditions. The function of this latter group of bacteria, which are characterized by ability to reduce nitrates to nitrites, is unknown. The same authors in a later paper (8) found the predominant organisms in the rhizosphere to consist of non-sporing rods with

well pronounced ammonifying capacity, and of mycobacteria. Spore-formers comprised an insignificant proportion of the rhizosphere population, though some rise was apparent towards the end of the vegetation period of the plants.

Further information as to predominant bacterial types developing about roots of growing plants has been given by Starkey (15) from microscopic observations of the rhizosphere. Employing the buried slide technique, he observed that the bacteria occurring in greatest abundance about root hairs, fungus filaments, and decomposing organic matter were small, coccoid cells. Longer rods were detected, but spore-formers were seldom encountered.

Studying the effect of organic soil amendments on the microflora of wheat roots, Thom, Clark, Fierke, and Fellows (17) found corynebacteria to be the most numerous types when either chicken manure or chopped green alfalfa was added to soil, though certain differences in physiological properties of the predominant organisms were observed under the two conditions. The observations were concerned with the influence of soil treatment rather than with the effect of the plant in modifying the soil microflora.

Experimental

The present report embraces qualitative studies of the bacterial flora of the rhizosphere of red clover, mangels, oats, tobacco, corn, and flax to note changes exerted by the various crops on the relative incidence of types present in control soils. In addition, comparative studies were made of the rhizosphere of certain varieties of tobacco and flax resistant and susceptible respectively to soil-borne fungous diseases.

For the analysis of field samples, blocks of soil about the plants were carefully dug up and brought to the laboratory. After removal of above-ground portions of the plants, the soil was gently broken to release the root system, which was added to sterile water together with adherent soil after gentle shaking to remove superfluous quantities. After thorough shaking suitable dilutions were prepared from the original flask for plating. The roots were later removed, contents of flask measured and evaporated to dryness to permit of the estimation of plate counts of rhizosphere soil on a dry weight basis. Controls consisted of corresponding composite soil samples distant from the plant; in the case of different plant varieties, e.g., tobacco, the rhizospheres of which were being compared, midway between rows. In the case of the greenhouse samples, plants were carefully removed from the pots and similarly treated. Controls in this case consisted of the remainder of the soil in the pot after thorough mixing.

Examination of rhizosphere and control soil was made on a quanti-qualitative basis following general procedures previously described (16). This involved a study of colonies growing on soil extract agar prepared according to Löhnis (10) without added energy material, and chosen as being as non-selective as possible, and hence most desirable for primary isolation purposes. While a "richer" medium would allow better colony development of many forms, this was considered a distinct disadvantage in view of the antagonisms

possible in plate cultures leading to the suppression of other forms which might otherwise appear, even as small colonies.

From representative plates, 60 to 100 colonies were picked, representing all on a plate or a sector, and stab cultures made to soil extract semi-solid medium (containing 0.02% K_2HPO_4, 0.01% yeast extract and 0.3% agar). The use of this medium assured the survival of 93% of the transfers, many from pin-point colonies. Obvious actinomycetes were not included. The present report is based on data from a study of 1,191 cultures.

Morphological observations of predominant soil types had indicated the presence of a large proportion of pleomorphic organisms, which in the majority of cases showed affinity to the corynebacteria. It was also observed that whereas on the richer artificial media pleomorphism was quite pronounced, little or none was observed in sterilized soil, or in soil extract semi-solid medium. Classification of the strains isolated, therefore, was made (i) on the basis of the forms occurring in soil extract semi-solid medium and considered to represent more closely the morphology of the organisms in soil, and (ii) on the basis of additional observations on a variety of richer artificial media to differentiate forms with a view to noting more closely their taxonomic relationship. For this purpose nutrient agar and yeast peptone agar as employed by Topping (19, 20) in her soil flora studies were used. Tests for motility, staining reactions, and biochemical properties were included to aid in grouping.

Comparison of Rhizosphere and Control Soils

In Table II is presented a summary of morphological groups as observed in soil extract semi-solid medium, in addition to certain physiological groups, derived from a study of individual cultures from the rhizosphere of various cultivated plants. In contrast to Table I, which indicates little or no difference due to fertilizer treatment, Table II reveals certain striking effects exerted by the growing plant on the bacterial groups in the soil.

While different plants show variations, both in total numbers and in the incidence of different microbial groups, yet there is noted an unmistakable selective action characteristic of the rhizosphere of all plants studied. In all cases Gram-negative short rods are proportionately increased in the rhizosphere as compared with the control soil. On the other hand Gram-positive short rods, coccoid rods, and spore-forming bacteria are relatively less numerous in the rhizosphere than in soil more distant from the plant. In the case of Gram-variable rods, *Bact. globiforme* group, cocci, and long non-sporing rods, no definite rhizosphere effect is noted suggestive of group stimulation or depression due to plant growth.

From the standpoint of bacterial physiology, the findings indicate a more active bacterial flora in the rhizosphere than in the soil beyond the zone of influence of the plant. In the rhizosphere is found a higher percentage of forms developing well on nutrient agar, of liquefying types, and of those causing acid or alkaline reactions in dextrose. A noticeably greater percentage

TABLE I

COMPARISON OF BACTERIAL GROUPS IN SOILS OF DIFFERENT FERTILITY

——	Soil N (no fertilizer)	Soil X (farmyard manure)	Soil Y (mineral fertilizer)
Relative crop producing capacity			
Timothy	55.9	100.0	85.1
Mangels	8.9	100.0	86.5
Morphological groups (soil extract)	%	%	%
Short rods, Gram-pos.	29.6	30.5	24.5
Short rods, Gram-neg.	30.5	35.2	42.1
Short rods, Gram-var.	7.0	10.8	10.3
Bact. globiforme group	11.2	7.5	8.3
Coccoid rods, Gram-pos.	8.4	5.6	3.9
Cocci	5.6	3.3	2.4
Long rods, non-sporing	3.3	3.8	4.4
Spore-formers	4.2	3.2	3.9
Physiological groups	%	%	%
Growth on N. A. very slight or absent	53.5	58.7	66.1
Gelatin liquefaction	28.6	27.7	29.4
Nitrate reduction	40.4	43.7	30.4
Acid reaction in dextrose 1%	42.7	38.5	43.6
Alkaline reaction in dextrose 1%	21.6	16.9	15.7

of motile organisms is present in the rhizosphere, while the incidence of chromogenic types shows a very pronounced increase over that in the control soils.

The more detailed comparison of the tobacco, corn, and flax soils, based on more extended observation to note specially pleomorphic forms, is summarized in Table III. Further points of interest are brought out in the relative incidence of certain broad generic groups as affected by plant growth. Non-sporing, non-pleomorphic, rod-shaped bacteria are proportionately increased in the rhizosphere, particularly Gram-negative forms. On the other hand, the broad group of pleomorphic organisms, comprising well over half the forms in the control soils, are relatively less abundant.

Investigations of such workers as Jensen (2–4), Ørskov (11, 12), Krassilnikov (5), Topping (19), and Umbreit (21) have called attention to the occurrence in soil of appreciable numbers of organisms belonging to non-sporing genera of the order Actinomycetales, forms showing characteristically the properties of bacteria, though usually with a considerable degree of pleomorphism and in some cases with a tendency towards formation of slight mycelium. The taxonomy of the order is at present confused and the nomenclature for what are apparently similar groups most varied. The classification of soil forms is hindered, not only by lack of sufficient study, but also because many soil species do not appear to fit into schemes of classification based largely on the more "classical" parasitic forms.

TABLE II

INCIDENCE OF BACTERIAL GROUPS IN RHIZOSPHERE AND CONTROL SOILS (A)

Classification	Rotation plots (N)				Tobacco soil			Corn soil		Flax (pot expt.)		
	Control	Rhizosphere			Control	Rhizosphere		Control	Rhizo.	Control	Rhizosphere	
		Clover	Mangels	Oats		RH. 211	CH. 38				Bison	Novelty
Plate count (millions)	48.6	254.8	108.2	118.1	94.7	269.2	505.4	28.2	389.2	98.3	439.9	2751.3
Morphology, Soil ex. semi-solid	%	%	%	%	%	%	%	%	%	%	%	%
Short rods, Gram-pos.	19.7	8.7	10.8	13.6	23.3	20.0	16.1	23.1	22.2	7.7	6.1	1.4
Short rods, Gram-neg.	30.3	55.1	43.1	45.5	13.3	43.3	45.2	26.9	51.1	15.4	36.4	59.1
Short rods, Gram-var.	16.7	15.9	15.4	19.7	11.7	6.7	17.7	5.8	6.7	7.7	7.1	14.1
Bact. globiforme group	7.6	1.5	10.8	1.5	13.3	10.0	16.1	7.7	4.4	1.9	16.2	1.4
Coccoid rods, Gram-pos.	13.6	5.8	10.8	10.6	18.3	10.0	4.8	21.2	13.3	53.8	32.3	16.9
Cocci	1.5	1.5	3.1	3.0	1.7	3.3	0.0	0.0	0.0	7.7	0.0	1.4
Long rods, non-sporing	4.6	8.7	6.2	3.0	6.7	5.0	0.0	5.8	2.2	0.0	1.0	5.6
Spore-formers	6.1	2.9	0.0	3.0	10.0	1.7	0.0	9.6	0.0	5.8	1.0	0.0
Physiology												
Good growth, nutrient agar	33.3	56.5	61.5	45.5	35.0	75.0	66.1	48.1	57.7	36.5	78.8	85.9
Chromogenic types	12.1	29.0	29.2	21.2	1.7	26.7	29.0	3.8	33.3	7.7	29.3	25.4
Motile forms	—	—	—	—	13.3	31.7	25.8	13.5	24.4	26.9	36.4	64.8
Liquefying (1 wk.)	—	—	—	—	15.0	16.7	41.9	23.1	24.4	42.3	54.6	70.4
Inhibited, 1% dextrose	42.4	27.5	20.0	22.7	30.0	25.0	25.8	11.5	8.9	5.8	1.0	0.0
Acid reaction, dextrose	39.4	42.1	53.8	43.9	36.7	38.3	48.4	56.7	57.8	50.0	67.7	81.7
Alk. reaction, dextrose	6.1	15.9	16.9	21.2	16.7	21.7	17.7	9.6	17.8	3.9	9.1	12.7

TABLE III

INCIDENCE OF BACTERIAL GROUPS IN RHIZOSPHERE AND CONTROL SOILS (B)

Classification	Tobacco soil			Corn soil		Flax (pot expt.)		
	Control	Rhizosphere		Control	Rhizo.	Control	Rhizosphere	
		RH. 211	CH. 38				Bison	Novelty
Plate count (millions)	94.7	269.2	505.4	28.2	389.2	98.3	439.9	2751.3
Non-pleomorphic	%	%	%	%	%	%	%	%
Cocci	1.7	1.7	0.0	0.0	0.0	1.9	0.0	0.0
Rods, spore-forming	10.0	1.7	0.0	9.6	0.0	5.8	1.0	0.0
Rods, non-sporing, total	21.6	41.7	40.3	28.8	44.4	11.5	24.2	54.9
Rods, non-sporing, Gram-pos.	18.3	20.0	20.9	21.2	24.4	5.8	10.1	18.3
Rods, non-sporing, Gram-neg.	3.3	21.7	19.4	7.5	20.0	5.8	14.1	36.6
Rods, non-sporing (pct. Gram-pos.)	(84.6)	(45.8)	(52.0)	(73.3)	(55.0)	(50.0)	(41.7)	(33.3)
Rods, non-sporing (pct. motile)	(38.5)	(48.0)	(41.7)	(6.7)	(30.0)	(33.3)	(50.0)	(66.7)
Rods, non-sporing (pct. chromogenic)	(0.0)	(32.0)	(28.0)	(13.3)	(53.0)	(0.0)	(41.7)	(26.2)
Pleomorphic								
Total (Proactinomycetaceae)	65.0	55.0	58.1	53.9	48.9	80.7	74.5	45.1
Acid-fast, non-motile, Gram-pos. (*Mycobacterium*)	8.3	0.0	1.6	1.9	2.2	3.8	0.0	0.0
Non-acid-fast, non-motile (*Corynebacterium*)								
Total	55.0	45.0	45.2	42.3	35.6	57.7	50.5	16.9
Gram-pos.	43.3	33.3	38.7	40.4	24.4	44.2	42.4	8.5
Gram-neg.	11.7	11.7	6.5	1.9	11.1	13.5	8.1	8.5
Non-acid-fast, motile (*Mycoplana*)								
Total	1.7	10.0	11.3	9.6	11.1	19.2	24.2	28.2
Gram-pos.	1.7	1.7	6.5	9.6	8.9	13.5	13.1	5.6
Gram-neg.	0.0	8.3	4.8	0.0	2.2	5.8	11.1	22.6
Non-acid fast (pct. Gram-pos.)	(81.9)	(63.7)	(80.0)	(96.3)	(71.4)	(75.0)	(74.3)	(35.5)
Non-acid-fast (pct. motile)	(2.9)	(18.2)	(20.0)	(18.4)	(23.8)	(25.0)	(32.4)	(61.3)
Non-acid-fast (pct. chromogenic)	(2.9)	(24.2)	(31.4)	(0.0)	(19.0)	(10.0)	(24.3)	(22.6)
Non-acid-fast (pct. liquefy.)	(14.7)	(24.2)	(54.3)	(22.2)	(23.8)	(52.5)	(56.8)	(54.8)
Non-acid-fast (pct. reduce NO_3)	(47.1)	(30.3)	(42.8)	(22.2)	(18.0)	(50.0)	(14.9)	(41.9)

Pleomorphic types considered to fall within the family Proactinomycetaceae following Jensen's (2) classification of the Actinomycetales, were found to comprise a large proportion of the bacteria in the control soils. It was found that clear-cut distinction of strains on the basis of such properties as slight mycelium formation, acid fastness, or reaction to the Gram stain is not entirely satisfactory in the separation of forms occurring in soil. Jensen (2) has already suggested that the transition from *Proactinomyces*, the criterion for which genus is the formation of initial mycelium, to *Mycobacterium* and *Corynebacterium* is very gradual, while Topping (19), Ørskov (12), and Umbreit (21) have also stressed the difficulty of definite distinction between these groups on the basis of slight mycelium formation. In the soils studied, mycelium formation was observed with relatively few strains and in the absence of other pronounced features distinguishing this group, they were classified, for the present purpose, with the acid-fast group (mycobacteria) or the non-acid-fast group (corynebacteria).

Non-acid-fast forms comprised the overwhelming majority of the proactinomycetes in the soils examined. These organisms exhibited characteristic "snapping" division in the rod stage and showed a tendency, particularly on richer artificial media, towards the production of irregular, swollen forms, often slightly branched or showing formation of buds or "sprouts". In older cultures, and more especially in soil extract media, the organisms appeared more predominantly as coccoids, arising from a process of fragmentation. Comparison of these forms, which showed closest affinity to the genus *Corynebacterium*, indicated that many apparently closely related strains differed in their reaction to the Gram stain and with respect to motility. Since the generic term *Corynebacterium* is usually reserved for non-acid-fast, non-motile, Gram-positive forms, the taxonomy of motile or Gram-negative forms may well be a matter of dispute. Some provision appears necessary for motile soil forms related to corynebacteria, the presence of which has been also stressed by Topping (19) and Ørskov (12). For the present purpose, non-motile forms are classified as *Corynebacterium*, with recognition of both Gram-positive and Gram-negative types, while motile forms are considered to show closest affinity to the genus *Mycoplana* Gray and Thornton, to include likewise both Gram-positive and Gram-negative forms. Whether this separation of motile from non-motile forms is preferable to the inclusion of both forms in the genus *Corynebacterium* will doubtless await further investigation, though some support of the latter alternative is given by our finding of occasional motile strains of the *Bacterium globiforme* group. The characteristic *Bact. globiforme* is now believed by us to represent a special group of the corynebacteria with distinctive cultural and physiological properties. In the present comparison of control soils and rhizosphere, where formal classification and identification of species were regarded as less important than the grouping of organisms according to certain morphological and physiological characters, it was found of advantage to consider motility and Gram reaction in differentiating between strains.

As noted from Table III the relative incidence of proactinomycetes as a whole is lower in the rhizosphere than in the control soils. Within the broad group, however, a selective action by the plant is apparent. The largest group in the control soils consists of non-acid-fast, non-motile forms regarded as corynebacteria. This group appears to correspond to organisms found by Jensen (3) to comprise an important group of organisms in Australian soils, and are doubtless to be included with the "mycobacteria" reported by Krassilnikov (5) to be abundant in Russian soils. This group showed a relative depression in the rhizosphere. On the other hand, motile forms, provisionally grouped as *Mycoplana*, appeared to be preferentially stimulated in the rhizosphere of all plants studied.

With pleomorphic as well as non-pleomorphic bacteria, Gram-positive forms were relatively less numerous in the rhizosphere than in controls, while chromogenic forms were conspicuously more abundant in the rhizosphere. Proactinomycetes with liquefying capacity were rather more abundant in the rhizosphere, though nitrate-reducing forms appeared to be slightly less numerous relatively as compared with the control soils.

Rhizosphere of Resistant and Susceptible Plants

In comparative quantitative studies of the rhizosphere of certain varieties resistant and susceptible respectively to soil-borne pathogens, Timonin (18), working in this laboratory, noted higher numbers of bacteria, and to a less extent, of fungi, in the case of the susceptible than of resistant varieties. This was observed in both greenhouse and field tests with flax susceptible to wilt (*Fusarium lini*), and with tobacco susceptible to black root rot (*Thielaviopsis basicola*). With actinomycetes no difference was observed. Since the tests were made with uninfected plants, the differences observed were believed to be due to inherent differences in physiological function, making in the case of susceptible plants, conditions somewhat more favourable for general bacterial development.

Qualitative surveys of the bacterial flora of the rhizosphere which included comparisons of resistant (Bison) and susceptible (Novelty) flax, and resistant (RH. 211) and susceptible (CH. 38) tobacco, are partly summarized in Table IV. Morphological classification according to forms developing in soil extract semi-solid medium showed differences in the relative incidence of three groups of organisms. Gram-negative short rods were relatively more abundant, and coccoid rods and spore-formers less abundant, in the rhizosphere of the susceptible than of the resistant varieties of plants. As noted above (Table II) the same trend was observed with respect to rhizosphere and control soil. These findings, taken in conjunction with the quantitative data, suggest that the susceptible plants studied exerted a greater "rhizosphere effect" than did corresponding resistant varieties.

The more detailed differentiation showed few significant differences. Non-motile, non-pleomorphic short rods, and motile, pleomorphic rods (*Mycoplana?*) showed a slightly higher incidence in the rhizosphere of susceptible plants.

TABLE IV

BACTERIA IN RHIZOSPHERE OF RESISTANT AND SUSCEPTIBLE PLANTS

Classification	Flax (field)		Flax (pots)		Tobacco (field)	
	Bison (R)	Novelty (S)	Bison (R)	Novelty (S)	RH. 211 (R)	CH. 38 (S)
Plate count (millions)	13.1	18.7	439.9	2751.3	269.2	505.4
Morphology—Soil ex. semi-solid	%	%	%	%	%	%
Gram-neg. short rods	32.2	44.4	36.4	59.1	43.3	45.2
Coccoid rods, Gram-pos.	10.2	6.3	32.3	16.9	10.0	4.8
Spore-formers	16.9	4.8	1.0	0.0	1.7	0.0
Detailed differentiation (non-sporing types)						
Non-pleomorphic						
Cocci	1.7	0.0	0.0	0.0	1.7	0.0
Rods, non-sporing, total	22.1	23.9	24.2	54.9	41.7	40.3
Rods, non-sporing, Gram-pos.	5.1	4.8	10.1	18.3	20.0	20.9
Rods, non-sporing, Gram-neg.	17.1	19.1	14.1	36.6	21.7	19.5
Rods, non-sporing, motile	6.8	6.3	12.1	36.6	20.0	16.1
Rods, non-sporing, non-motile	15.3	17.6	12.1	18.3	21.7	24.2
Pleomorphic						
Acid-fast, non-motile, Gram-pos.	1.7	7.9	0.0	0.0	0.0	1.6
Non-acid-fast, non-motile, total	39.0	46.0	50.5	16.9	45.0	45.2
Non-acid-fast, non-motile, Gram-pos	23.4	28.6	42.4	8.5	33.3	38.7
Non-acid-fast, non-motile, Gram-neg.	13.6	17.4	8.1	8.5	11.7	6.5
Non-acid-fast, motile, total	13.5	14.3	24.2	28.2	10.0	11.3
Non-acid-fast, motile, Gram-pos.	8.4	9.5	13.1	5.6	1.7	6.5
Non-acid-fast, motile, Gram-neg.	5.0	4.8	11.1	22.6	8.3	4.8
Physiological						
Motile organisms	28.8	30.1	36.4	64.5	31.7	25.8
Chromogenic forms	5.1	6.3	29.3	25.4	26.7	29.0
Liquefying	15.3	15.9	54.6	70.4	16.7	41.9
Acid reaction, dextrose	45.7	46.0	67.7	81.7	38.3	48.4
Nitrate-reducing	30.5	31.7	21.2	49.3	35.0	41.9

Physiological tests pointed, if anything, to a somewhat more active microflora in the rhizosphere of susceptible plants.

Although these preliminary findings by no means prove, yet they suggest the possibility, that resistance to certain disease may be linked up with a selective action of root excretions upon the saprophytic soil microflora, thus favouring types which may be more, and in other cases less, antagonistic (directly or indirectly) towards pathogenic organisms.

Conclusion

The results as a whole emphasize the importance of enlarging our knowledge of the qualitative nature of the soil micro-organisms and particularly those types the functions of which are still unknown, but which appear to comprise a large proportion of the micro-population of arable soils. Up to the present

the study of processes, and incidentally that of specialized groups of organisms concerned in these processes, has received more attention than the objective study of soil organisms on a non-selective basis. It is believed, however, that more study from the qualitative side is essential to help complete our knowledge of the types indigenous in soil and the changes in the trend of population in the rhizosphere. It is here where not only the main interaction occurs between soil micro-organisms and the growing plant, but where the action of various soil-borne pathogens or toxic factors occurs to complicate and accentuate asociative or antagonistic influences.

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CROSSING AND SELFING STUDIES WITH PHYSIOLOGIC RACES OF OAT STEM RUST[1]

By Thorvaldur Johnson[2] and Margaret Newton[3]

Abstract

Evidence has been obtained through crossing and selfing studies that some physiologic races of oat stem rust contain both homozygous and heterozygous lines, the latter when selfed tending to produce races more virulent than the selfed race. In crosses between races, the less virulent characteristics are generally dominant so that the hybrid race is often identical with the less virulent of the two parent races. Selfing studies and crosses therefore indicate that the more virulent characteristics of oat stem rust are recessive traits. Reciprocal crosses between physiologic races suggest that the cytoplasm, as well as the nucleus, contributes to the inheritance of certain pathogenic characters.

In crosses between races of normal (red) and orange uredial colour, the red colour has invariably proved dominant, the progeny being indistinguishable in colour from the normal parent.

Two selfing studies of Race 3 carried out with the same telial material at an interval of more than four years have provided evidence of a genetic change presumably brought about through the ageing of the teliospores. In the first selfing, no abnormalities were observed in the infections on the barberry. In the second selfing, about half of the infections developed pycnia and pycnial nectar, while the remainder appeared as small, round, necrotic areas showing no indication of even rudimentary pycnial formation.

Introduction

One of the difficulties encountered in inheritance studies with physiologic races of stem rust is the scarcity of characters whose inheritance can be studied. The urediospores of the different races are, for all practical purposes, identical morphologically. An inheritance study based on morphological characteristics is therefore out of the question. While differences in spore colour exist among the races collected in nature, these are, in general, so slight as to be almost imperceptible. It is only when races of decidedly abnormal spore colour arise, such as the orange races described in the present paper, that the inheritance of spore colour can be studied. Such colour variants have, however, not yet been discovered in the field and are but rarely encountered in the laboratory.

In the absence of definite morphological differences between races, inheritance studies must perforce be limited largely to pathogenic characteristics which find expression in the so-called "infection types" on seedling leaves of cereals. The fact that an infection type of a physiologic race is relatively constant on a given host variety grown under given conditions makes it permissible to regard it as a character of the rust. Its inheritance may then be investigated in crosses and selfing studies. A race of oat stem rust produc-

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ing a 2 infection type on the oat variety, White Tartar, may be crossed with another race producing a 4 infection type, the object being to discover the infection type of the hybrid race on White Tartar. Other infection types produced on other host varieties may be studied in a similar way. In selfing studies with races, it is possible to discover whether they are homozygous or heterozygous for each of the various infection types they produce on the differential oat varieties.

The work reported in the present paper was undertaken with the object of studying the breeding behaviour of known physiologic races of *Puccinia graminis Avenae* Erikss. and Henn., first, through the selfing of each race and, second, through crosses between the different races. The selfing studies were expected to furnish an indication of what might happen in nature when races infect barberries and spread from them. The crossing studies were expected to yield information on the pathogenic characters of the F_1 hybrid races in comparison with those of the parent races. If this program could have been carried through to completion for all the races known at present, it might have been possible to predict to a certain extent the pathogenic characteristics of new races that might be expected to arise, in nature, as new combinations on the barberry.

During the course of this work, however, certain obstacles have intervened to prevent its completion, particularly the sparseness of teliospore formation in races 1,2, and 5, and the difficulty of inducing greenhouse-formed teliospores of certain races to germinate. Nevertheless, selfing studies have been conducted, though sometimes on a limited scale, with seven of the twelve races known at the present time, and crosses have been made between several physiologic races. Although the work is not yet by any means complete, it is the opinion of the writers that sufficient evidence has been secured to warrant some generalization on the inheritance of certain of the pathogenic characteristics of oat stem rust.

Selfing Studies

The procedure followed and the technique employed in the selfing studies here reported have been described in detail elsewhere (7). Briefly stated, it consisted in (i) developing teliospores of pure cultures of the various physiologic races, (ii) inducing the teliospores to germinate, (iii) infecting the barberry with the sporidia, (iv) infecting oat seedlings with the aeciospores, and (v) identifying the physiologic races present in the resulting uredial cultures.

To gain an idea of the numerical distribution of physiologic races in the progeny of any selfing, aecial cups were picked off singly, at random, from numerous aecial pustules, crushed, and transferred individually to seedling leaves of a susceptible oat variety. In each selfing study this method was usually supplemented by one or two mass transfers of aecia from a large number of aecial pustules, the object of which was to ascertain whether all the races present on the barberry were represented in the random collection of aecia. The identification of the physiologic races was accomplished by

means of the three differential varieties White Tartar, Richland, and Joanette Strain, originally selected by Stakman, Levine, and Bailey (10) and by Bailey (1). On these varieties the different physiologic races produce characteristic infection types, designated by Bailey as 1, 2, 3, 4, and x (Table I). These infection types may be regarded as an expression of either the pathogenicity of the rust or the reaction of the host plant. Viewed in terms of host reaction, types 1 and 2 are regarded as indicating resistance, owing to the smallness of their pustules and the presence of necrosis and chlorosis, while type 4 with its large pustules is regarded as indicating susceptibility. In the text, types 1 and 2 are frequently referred to as small pustules and type 4 as large pustules to avoid constant mention of these infection types by numerals. The infection types chiefly dealt with in this paper—types 1, 2, 4, and x—are shown in Plate I, Fig. 9.

TABLE I

MEAN INFECTION TYPES PRODUCED BY PHYSIOLOGIC RACES OF *P. graminis Avenae* ON SEEDLING LEAVES OF OAT DIFFERENTIAL VARIETIES

Physiologic race	White Tartar	Richland	Joanette Strain
1	2+	1	1±
2	2++	1+	4
3	4−	1	1−
4	4	4+	1
5	2++	1++	x±
6	4+	4+	4+
7	4	2=	4−
8	2	4	3++
9	2±	x+	4
10	2	4	x+
10*a**	2+	4	1±
11	2	4	1=
12**	4=	1++	x+

* *Race 10a resembles the Race 11 described by Dr. K. Hassebrauk (Arb. biol. Reichs. 22 : 479-482. 1938) but as it occasionally produces an* x *type on Joanette Strain it is regarded as a variant of Race 10.*

** *The writers are indebted to Dr. E. C. Stakman and Dr. M. N. Levine for assigning a number to this race. They originally numbered it 11, but owing to the fact that Dr. Hassebrauk in the meantime had given this number to another race they suggested that the number of the new race be changed to 12.*

THE SELFING OF RACES COLLECTED IN THE FIELD

The earliest record of an artificial selfing of a known race of oat stem rust is a selfing of Race 8 reported by Gordon and Welsh (3). In a study of cultures arising from 16 separate aecial cups and four mass transfers of aecia, Race 8 occurred 15 times, Race 6 occurred seven times, and Race 7 once.

The selfing studies carried out by the present writers with races collected in nature are summarized in Table II. While some of these studies were

TABLE II

THE SELFING OF RACES OF *P. graminis Avenae* COLLECTED IN THE FIELD

Race selfed	Races occurring in F_1 population	Remarks on genotype of race selfed
2	2 (23 cultures)	Homozygous
2	2 (3 cultures)	
3	3 (15 cultures) 4 (8 cultures) 12 (1 culture)	Heterozygous for infection type on Richland
5	2 (11 cultures) 5 (91 cultures) 10 (2 cultures) 12 (2 cultures)	Heterozygous for infection type on Joanette Strain
7	7 (43 cultures)	Homozygous
8	6 (2 cultures) 8 (4 cultures)	Heterozygous for infection type on White Tartar
8	6 (6 cultures) 8 (8 cultures)	Heterozygous for infection type on White Tartar
10*a*	10*a* (25 cultures)	Homozygous

conducted on a small scale, they nevertheless throw some light on the genotypic constitution of the six races selfed. The selfing of two separate cultures of Race 2 suggested that this race is homozygous for pathogenic characters, at least for those that can be distinguished by the reactions of the differential oat varieties used. The selfing of Race 7 and 10*a* also indicated that they were homozygous. Races 3, 5, and 8, however, are clearly heterozygous in respect to their infection types on one or the other of the three differential hosts used.

The pathogenic character of the progeny of the three heterozygous races —Races 3, 5, and 8—is of some interest. The selfing of Race 3 gave rise chiefly to Races 3 and 4. The latter differs from the former only by the infection type on the variety Richland, which is susceptible instead of resistant to this race. In this selfing, therefore, a race to which Richland is resistant has produced a race to which this variety is susceptible. In other words, the small-pustule type appears to be dominant to the large-pustule type. Similarly, in the selfing of Race 8, a race which produces small pustules on White Tartar, there arose a number of cultures of Race 6, a race which produces large pustules on White Tartar, as happened also in the above-mentioned selfing of this race by Gordon and Welsh. This result definitely suggests the dominance, on White Tartar, of the smaller type of pustule.

The selfing of Race 5 would suggest that this race, like Race 2, is homozygous for its infection types on White Tartar and Richland but is heterozygous for the infection type on Joanette Strain. The occurrence of two cultures of

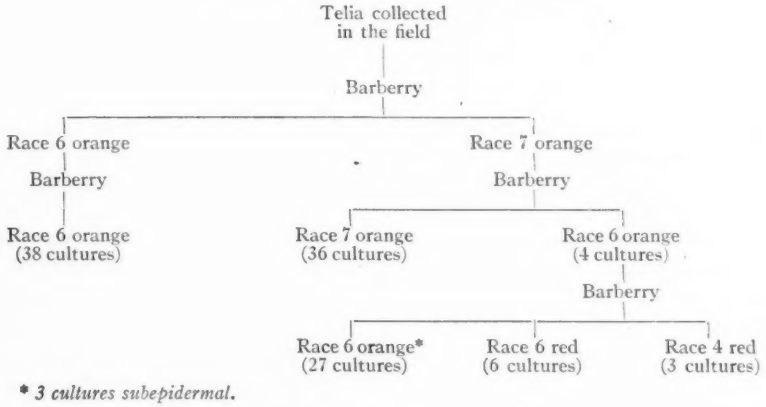
Race 10 and two of Race 12 in the total progeny of 106 cultures may be the result of either a slight admixture of some other race in the selfed culture or a slight amount of accidental intermixture of the pycnial nectar of other races at the time of selfing.

The Selfing of Races of Abnormal Uredial Colour

In addition to selfing studies with races collected in the field, similar studies have been conducted with certain cultures of abnormal uredial colour. As already reported by Gordon (2), two such cultures originated in 1931 from aecia on a barberry plant artificially inoculated with sporidia of teliospores gathered from field plots at Winnipeg in the previous autumn. These cultures, identified as Races 6 and 7, produced uredia described as "ochraceous orange", according to Ridgway's Colour Standards. Selfing studies carried out with these races by the present writers are summarized in the flowsheet.

FLOWSHEET

THE SELFING OF RACES OF ABNORMAL COLOUR



* *3 cultures subepidermal.*

From these studies, it appears probable that Race 6 is homozygous, as 38 cultures from aecia collected at random were all identified as Race 6 orange. Race 7, however, is heterozygous, 36 cultures being identified as Race 7 orange and 4 cultures as Race 6 orange. Subsequently a selfing study of one of the last-mentioned cultures (Race 6 orange) resulted in a progeny composed of 27 cultures of Race 6 orange, 6 cultures of Race 6 red, and 3 cultures of Race 4 red. The unexpected appearance of cultures of normal colour may conceivably be due to mutation, but is more likely the result of an undetected admixture of a race of normal uredial colour. In connection with the selfing, it may be mentioned that three of the orange cultures failed completely to rupture the epidermis of the seedling leaves, a phenomenon never before noted in stem rust of oats although of not infrequent occurrence

in selfed races of stem rust of wheat (4). In relation to the selfing of Race 7 orange, it may be noted that this race is heterozygous whereas, as already pointed out, a field culture of Race 7 gave evidence of being homozygous. The heterozygosity of Race 7 orange resembles that of Race 3 (mentioned above) in that the small-pustule type on Richland is dominant to the large-pustule type. The existence of two genotypically different strains of Race 7 makes it clear that phenotypic similarity, as expressed by the reactions of differential varieties, is not necessarily an index of genotypic identity.

Crosses Between Physiologic Races

Crosses were made between physiologic races of *P. graminis Avenae* with the purpose of comparing the pathogenic characteristics of the F_1 hybrid races with those of the parent races used in the crosses. Such a comparison can be readily obtained by noting the infection types produced by each of the parent races and their hybrid race on the three differential varieties, White Tartar, Richland, and Joanette Strain. If crosses could be made between enough of the races it should be possible to arrive at some generalization as to the inheritance of the pathogenic characteristics expressed through the reaction of these varieties. It should, for example, be possible to determine, in any cross between a race producing small pustules on Richland and a race producing large pustules, whether one of these types is dominant over the other, or whether an intermediate infection type results. If crosses could be made between all of the races differing in this respect and it were found that all hybrid cultures produced a similar infection type, a general rule, applicable to the known races of oat stem rust, might be laid down for the inheritance of this characteristic. The same line of reasoning applies to crosses between races differing in infection type on other differential varieties. As crosses have not yet been made between all of the known races, any generalization made at present must be regarded as more or less tentative.

The methods employed in making crosses need only be described here in brief outline as they have been published previously in detail (6–8). One or more barberry plants are infected sparsely with each of the two races to be crossed. If the pustules resulting from the infection are thinly distributed over the leaves, a considerable proportion will be haploid and hence will fail to develop aecia. When it seems evident that these pustules will not produce aecia if left alone, nectar is transferred from one pustule of one race to several pustules of the other race. As a result of these transfers of pycniospore-bearing nectar, aecia are usually formed in about one-half of the pustules. The aecia thus produced are then used to inoculate oat seedlings of a susceptible variety, after which the physiologic races present in the resulting cultures are identified by means of the differential varieties previously mentioned. The methods described above are illustrated in Fig. 1 by some actual crosses between Races 7 and 10*a*.

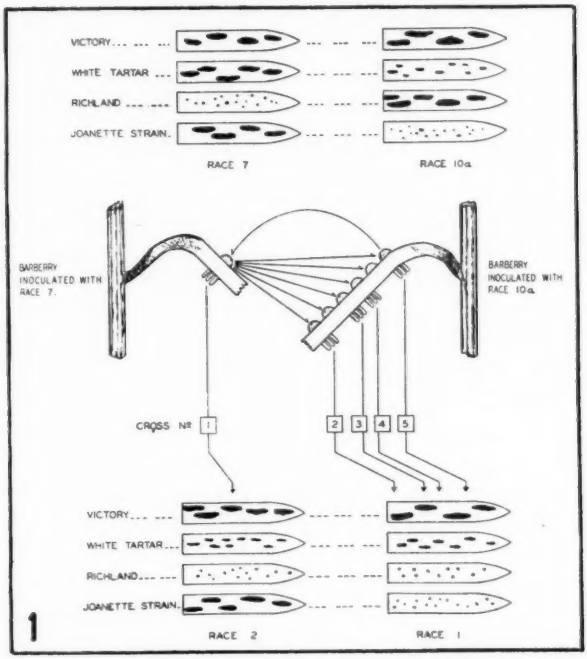


FIG. 1. *A diagrammatic representation of five actual crosses between Race 7 and Race 10a together with the infection types of the parent races and the F_1 hybrid races on the differential varieties. The arrows indicate the direction of transfer of pycnial nectar.*

INHERITANCE OF INFECTION CHARACTERISTICS ON WHITE TARTAR AND RICHLAND

Crosses between Races 2 and 7

These two races differ only by their infection types on the variety White Tartar, on which Race 2 produces small pustules and Race 7 large. The object of crossing them was therefore primarily to discover the infection type of the hybrid race on White Tartar. As each of the parent races was homozygous for the character in question, it was not necessary to make many crosses to decide this point. The results of crosses made in both directions are presented in Table III, A.

As the progeny of the crosses consisted of Races 2 and 5, which produce small pustules on White Tartar, it is evident that the small-pustule type of the Race 2 parent is dominant to the large-pustule type of the Race 7 parent (Fig. 2).

A similar dominance of the small-pustule type on White Tartar was revealed in the previously-mentioned selfing of two different cultures of Race 8, which produces a small type of pustule on this variety. The presence of Race 6,

which produces large pustules in the progeny of both cultures, shows that this type must be recessive to the small-pustule type.

Crosses between Race 5 and Race 7 Orange

Race 5 produces identically the same infection types on White Tartar and Richland as does Race 2, that is, small pustules. Races 5 and 7 differ, therefore, only by their infection types on White Tartar, Race 5 producing the small- and Race 7 the large-pustule type. As in the crosses between Races 2 and 7, the small type of pustule is dominant to the large, so that most of the F_1 progeny (5 out of 7 crosses) produces, on White Tartar, the small pustules characteristic of Race 5. In two of the seven crosses (Table III, B), however, the F_1 progeny was identified as Race 7. As each of these two cultures originated on the Race 7 side of a cross, they might be attributed to accidental

TABLE III

CROSSES BETWEEN RACES OF OAT STEM RUST

—	Races crossed	Number of crosses made	F_1 progeny
A	2* × 7	4	Race 2 (3 crosses) Races 2 and 5 (1 cross)
	7 × 2	3	Race 2 (3 crosses)
B	5 × 7 orange	3	Race 5 (3 crosses)
	7 orange × 5	4	Race 2 (2 crosses) Race 7 (2 crosses)
C	5 × 10*a*	1	Race 5 (1 cross)
	10*a* × 5	2	Race 1 (2 crosses)
D	5 × 6 orange	5	Race 5 (3 crosses) Race 10 (1 cross) Race 12 (1 cross)
	6 orange × 5	9	Race 2 (9 crosses)
E	5 × 8	4	Race 5 (2 crosses) Races 5 and 12 (1 cross) Race 12 (1 cross)
	8 × 5	2	Race 2 (1 cross) Race 10 (1 cross)
F	6 orange × 10*a*	2	Race 8 (2 crosses)
	10*a* × 6 orange	2	Race 10*a* (2 crosses)
G	7 orange × 10*a*	16	Race 2 (7 crosses) Race 8 (9 crosses)
	10*a* × 7 orange	10	Race 1 (6 crosses) Race 10*a* (4 crosses)

** The race placed first is the race on whose haploid pustules aecia are developed as a result of a transfer to them of pycniospore-containing nectar of the other race.*

selfing of Race 7 orange were it not for the fact that they produce red uredia, a result that suggests that they are in reality the product of crossing. If so, they serve as further proof of the impurity of the culture of Race 5 used in these crosses (see discussion of selfing of Race 5).

Crosses between Race 5 and Race 10a

These two races differ by their infection types on Richland, on which Race 5 produces small and Race 10*a* large pustules. The three crosses made between these two races suggest that the small-pustule type of Race 5 is dominant, as all the hybrid cultures produced this type (Table III, C and Fig. 3).

Crosses between Race 5 and Race 6 Orange

These two races differ by their infection types on both White Tartar and Richland, varieties that are resistant to Race 5 and susceptible to Race 6. As 12 of the 14 crosses resulted in progeny with small pustules (Table III, D and Fig. 4), it appears probable that the small-pustule types of Race 5 are dominant to the large-pustule types of Race 6.

The occurrence of Races 10 and 12 in two of the crosses is interpreted as the result of the impurity of the Race 5 parent.

Crosses between Race 5 and Race 8

Race 8 produces exactly the same infection types on White Tartar and Richland as does Race 10*a*. The crosses between Races 5 and 8 would, therefore, be expected to give results similar to the crosses between Races 5 and 10*a*, in which the small-pustule type of Race 5 on Richland proved dominant to the large-pustule type produced by Race 10*a*. In the crosses between Races 5 and 8, the small-pustule type of Race 5 did, in fact, show dominance to the large-pustule type of Race 8 in five of the six crosses (Table III, E). The occurrence of Race 10 in one cross is perhaps due to the impurity of the Race 5 parent. The unexpected occurrence of Race 12 in two of the crosses may have a similar explanation.

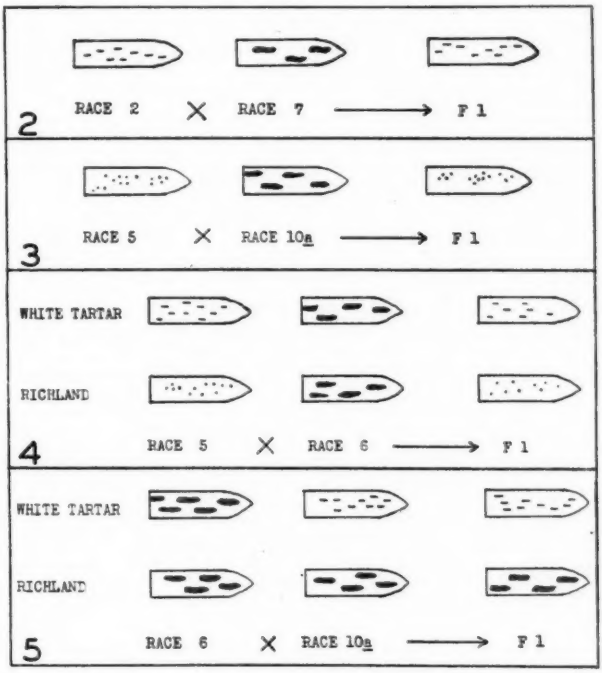
Crosses between Race 6 Orange and Race 10a

These two races differ by their infection types on White Tartar, on which Race 6 produces large and Race 10*a* small pustules. In the four crosses between these two races (Table III, F), the small-pustule type produced by Race 10*a* on White Tartar proved dominant to the large-pustule type produced by Race 6 (Fig. 5).

Crosses between Race 7 Orange and Race 10a

These two races differ by their infection types on White Tartar and Richland. Race 7 produces large pustules on White Tartar and small pustules on Richland, whereas Race 10*a* produces small pustules on White Tartar and large pustules on Richland. The results of crosses between these two races are shown in Table III, G.

The interpretation of the results of the crosses is complicated by the fact that Race 7 orange is heterozygous for its infection type on Richland. A previous selfing of this race (Flowsheet) produced, in addition to Race 7, a number of cultures of Race 6, which develops large pustules on Richland. It would therefore be expected, in any crosses with Race 10*a*, that about one-half of the F_1 cultures would produce large pustules on this variety, whereas, if Race 7 were homozygous, all of the F_1 progeny would produce small pustules. Actually 13 of the 26 F_1 cultures studied produced small pustules and 13 large pustules. The factor governing the small-pustule type of Race 7 on Richland is therefore dominant to that governing the large-pustule type of Race 10*a* on this host.



FIGS. 2–5. FIG. 2. *Infection types on White Tartar of Races 2 and 7 and their F_1 hybrid.* FIG. 3. *Infection types on Richland of Races 5 and 10a and their F_1 hybrid.* FIG. 4. *Infection types on White Tartar and Richland of Races 5 and 6 and their F_1 hybrid.* FIG. 5. *Infection types on White Tartar and Richland of Races 6 and 10a and their F_1 hybrid.*

The results of the crosses, with reference to White Tartar, are simpler to interpret as both parent races were homozygous for their infection types on this variety. All of the 26 F_1 cultures produced the small type of pustule, which proves that, for White Tartar, the small-pustule type of Race 10*a* is dominant to the large-pustule type of Race 7.

Inheritance through the Maternal Cytoplasm

In crosses between certain races of *P. graminis Tritici* Erikss. and Henn., previously described by the writers (7, 9), evidence was obtained that some pathogenic characteristics are inherited through the medium of the cytoplasm of the maternal parent race, that is to say, the race receiving a transfer of pycnial nectar. Where this type of inheritance is manifest, the races arising from the opposite sides of a cross are always different, whereas, if the chromosomes alone functioned, the races should be identical. An examination of Fig. 1 will show that this state of affairs exists in crosses between Races 7 and 10*a*, which produce different infection types on Joanette Strain. The races arising from the Race 7 side of a cross resemble Race 7 in that they produce large pustules on Joanette Strain, while the races arising from the Race 10*a* side of a cross resemble Race 10*a* in that they produce small pustules on this variety. This situation holds true not only for all crosses between Races 7 and 10*a* but apparently also for all crosses in which the two parent races differ in their infection types on Joanette Strain. In almost all such crosses there is a well marked tendency on the part of the hybrid race to resemble the maternal parent race in its infection characteristics on Joanette Strain. In the crosses reported in the present paper, this peculiarity has been noted in crosses between Races 7 and 10*a*, 5 and 7, 5 and 6, 5 and 8, 5 and 10*a*, and 6 and 10*a*. Consequently, in crosses between all these races, the same physiologic race never arises from a cross and its reciprocal cross. Owing to the infection type on Joanette Strain being different, the hybrid races of crosses made reciprocally are always identified as different physiologic races.

This phenomenon does not seem to be capable of explanation on a chromosomal basis, but can be readily explained if it is assumed that the pycniospore nucleus of the paternal race reaches the proto-aecium of the maternal race unaccompanied by its cytoplasm, which, in any case, is very limited on account of the minute size of the pycniospore. Each hybrid aeciospore would then receive from the paternal race only a nucleus, but from the maternal race a nucleus plus cytoplasm. This explanation, according to Lamb (5), "finds an entirely convincing cytological explanation" in his study of the initiation of the dikaryophase in *Puccinia Phragmitis* (Schum.) Körn.

The Inheritance of Urediospore Colour

The fact that, in several of the crosses already described, one race was orange while the other was normal (red) in urediospore colour provided opportunities of studying the inheritance of uredial colour as manifested in the F_1 generation. As the orange races, when selfed, produce uredia of their own colour, it is obvious that any red uredial cultures originating from the orange side of a cross between red and orange races are bound to be hybrids between the two races. The present paper describes crosses between Races 7 orange and 10*a* red, 7 orange and 5 red, 6 orange and 5 red, and 6 orange and 10*a* red. In these crosses, hybrid aecia arising from mycelia of the orange

races have invariably produced red uredial progeny, which proves not only that actual crossing took place but also that red spore colour is dominant to orange. A similar dominance of red to orange spore colour has previously been described for *P. graminis Tritici* (9).

The Effect of Ageing of Teliospores on the Infection Characteristics on the Barberry

In most of the selfing studies reported in this paper, the selfing was carried out within a few months of the formation of the teliospores. The selfing of Race 3, however, forms an exception in that two selfing studies were conducted with the same telial material, the first when the teliospores were about 6 months old, and the second when they were almost 5 years old. In the interval between the two selfings, the teliospores were kept in dry storage at a temperature of about 8 to 10° C. for 4 years and 138 days, namely, from May 15, 1934, to September 30, 1938. During this period of storage, it appears that some change took place in the spores, which resulted in abnormal infection characteristics on the barberry.

In the first selfing, no abnormalities were observed in the infections on the barberry. In the second selfing, however, it was noted that only about half of the infections developed pycnia and pycnial nectar; the remainder had the appearance of small, round necrotic areas frequently surrounded by a purple halo (Plate 1, Fig. 6). When the leaves were cleared with chloral hydrate and stained with acid fuchsin, the mycelia in these necrotic areas showed, in most instances, no indication of even rudimentary pycnial formation (Plate 1, Fig. 7).

A count of the normal and abnormal infections on four different barberry plants showed that nearly half of the infections were of the abnormal type: in a total of 308 infections, 170 were normal and 138 abnormal. These figures suggest that about half of the sporidia were deficient in some respect, so that the mycelia derived from them no longer possessed the ability to develop pycnia. This deficiency appeared to be in no way related to their sex. Both sexes were obviously present among the normal haploid pustules, as aecia formed readily from intermixture of pycnial nectar. That the deficiency was caused by some change in the teliospores during their long period of storage is suggested by the fact that no such abnormalities were noted in the first selfing.

Discussion

The object of the work described in the present paper was, as already stated, to study the inheritance of the pathogenic properties exhibited by physiologic races of *P. graminis Avenae* on the differential varieties used for their identification. While pathogenic properties are in reality physiologic characteristics they have nevertheless a concrete, visible expression, namely, the infection types produced on the leaves of the oat plant. These infection types, the visible manifestations of physiologic potentialities, may, owing to their relative constancy, be considered characters expressing the pathogenicity of the physiologic races.

The two methods of approach to this problem, viz., selfing of individual races and crosses between races, are complementary and yield much the same type of information. The complementary nature of the two methods may be illustrated by considering a definite example such as, for instance, the relation in selfing studies and crosses between the small and the large pustule types on the variety White Tartar. Selfing studies (Table II) have shown that races with small pustules may give rise to races with large pustules, as well as to races with small pustules, whereas races with large pustules have never been known to give rise to races with small pustules. These results therefore suggest the dominance of the small-pustule type. In crosses between Races 2 and 7, and Races 7 and 10*a*, the small-pustule type of Races 2 and 10*a* is dominant to the large-pustule type of Race 7 in the F_1 progeny. As far as the relationship of these two infection types is concerned, both crosses and selfing studies indicate the dominance of the small type of pustule.

Evidence obtained in a similar way suggests that the small type of pustule on Richland is dominant to the large type. This conclusion is derived from crosses between Races 7 and 10*a*, 5 and 10*a*, and 5 and 6, and from selfing studies with Race 3 (Table II) and Race 7 orange (Flowsheet), both of which produce small pustules on Richland but, when selfed, give rise to races with large as well as small pustules.

If, as seems quite possible, these facts are applicable to oat stem rust in general, they may help to throw some light on the role played by the barberry in the development of physiologic races. Owing to the dominance of the small-pustule types, it is always possible for the less virulent physiologic races, if they occur in a heterozygous state, to produce, through natural selfing, races of greater virulence on the newly developed rust-resistant varieties, most of which react to stem rust like either Richland or White Tartar. But, for exactly the same reason, natural crosses of the less virulent with the more virulent races are likely to produce hybrid races resembling the less virulent of the two races entering into a cross. It would seem, therefore, that there are two opposing processes at work, one of which, selfing, tends to bring into being strains of rust with greater virulence than the selfed parent race, while the other, crossing, tends to submerge the more virulent, recessive characteristics of the rust. Perhaps, in the long run, the two opposing processes will balance each other so that there may not be any significant increase in the virulence of oat stem rust through the medium of the barberry.

Nothing much can be said, at present, concerning the mechanism of the inheritance of the pathogenic properties discussed above. The presence of the phenomena of dominance and recessiveness suggests a chromosomal basis and a Mendelian inheritance, but in view of the small populations investigated in the selfing studies it is not possible to place this inheritance on a factorial basis. That the inheritance of pathogenic characteristics is not entirely chromosomal is shown by the apparent influence of the cytoplasm of the maternal race on the infection type on Joanette Strain. As a result, the infection type of the hybrid race on Joanette Strain closely resembles that of the

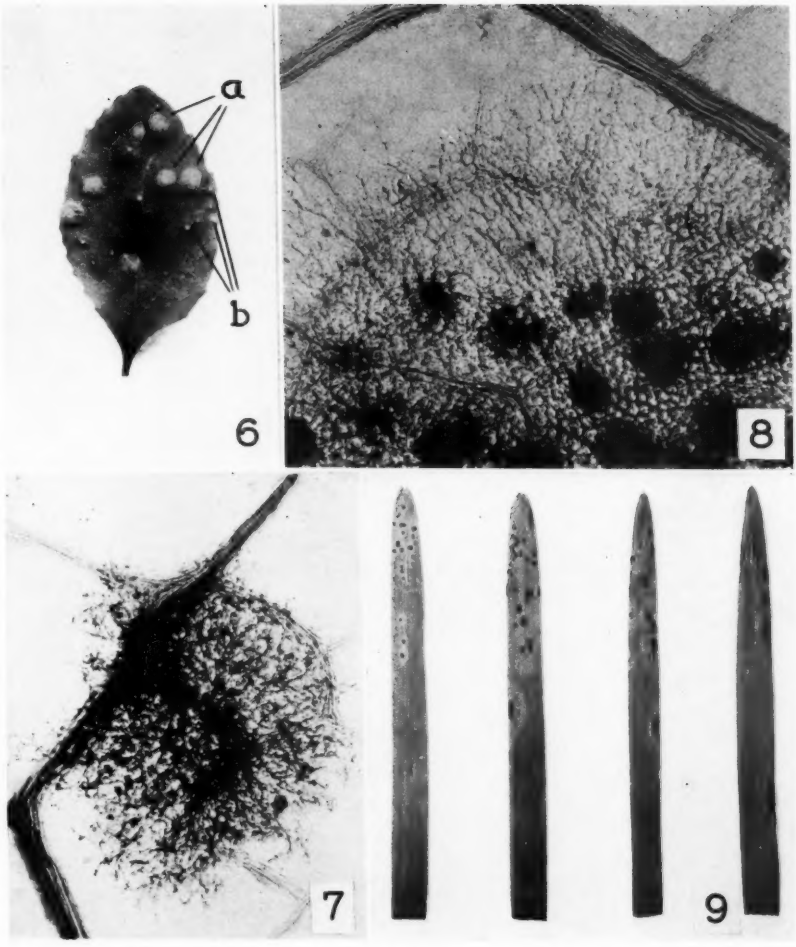


PLATE I. FIGS. 6–9. FIG. 6. *A leaf of Berberis vulgaris showing (a) normal infections (b) and abnormal infections produced by Race 3 after the teliospores had been kept in storage for more than 4 years.* FIG. 7. *Mycelium of an abnormal haploid pustule 24 days after inoculation.* ×650. FIG. 8. *Mycelium of a normal haploid pustule 24 days after infection, showing proto-aecia.* ×650. FIG. 9. *Infection types produced by P. graminis Avenae on seedling leaves of the differential oat varieties used for the identification of physiologic races. From left to ri ht: types 1, 2, x, 4.*

maternal parent race. Exactly the same phenomenon has been noted previously in crosses between physiologic races of *P. graminis Tritici* (7, 9), the influence of the cytoplasm there being discernible only in the infection types on the varieties Marquis and Kota.

In general, there is a rather close parallelism between the inheritance of pathogenic and other characters in *P. graminis Avenae* and *P. graminis Tritici*. In both rusts certain pathogenic properties are dominant to others; in both normal (red) spore colour is dominant to the atypical orange colour; and in both there is evidence of the influence of the cytoplasm on inheritance. While the effect of inbreeding on races of oat stem rust has not been studied extensively, there is some evidence that it leads to the development of strains with abnormal characteristics such as atypical colour and lack of vigour of sporulation. The appearance, after the third consecutive selfing (Flowsheet), of strains with uredia that failed to rupture the epidermis of the oat seedling leaf would suggest that, as in wheat stem rust, repeated selfings tend to lower the survival value of the rust.

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THE INTERRELATION OF LIPIDS IN THE BLOOD PLASMA OF WHITE LEGHORN COCKERELS[1]

BY ELDON M. BOYD[2] AND ELEANOR L. CLARKE[3]

Abstract

The blood plasma of 22 healthy, young, white leghorn cockerels was analysed by oxidative micromethods and found to contain the following mean lipid values, in milligrams per 100 ml. of plasma: total lipid 520, neutral fat 225, total fatty acids 361, total cholesterol 100, ester cholesterol 66, free cholesterol 34, phospholipid 155. In general, the amounts of the various lipids were proportionately related to the total lipid.

In recent communications, Boyd has shown that when values for the various lipids of blood plasma of man (3) and rabbits (4) are plotted against increasing values for total lipid, it is demonstrated that in normal animals a proportionate increase occurs in the value of each of the component lipids. During certain experiments recently performed in this laboratory, a group of some two dozen healthy, normal, white leghorn cockerels became available, and it appeared desirable to make a further study of the interrelation of plasma lipids in these birds belonging, as they do, to a class of vertebrates different from that usually experimented upon in studies of lipid metabolism. A differential lipid analysis of the blood plasma of cockerels has not been made with oxidative micromethods. The few studies of blood lipids in birds that have been done have been concerned chiefly with the effect of egg-laying (e.g., 9, 11) and in none of these has a complete analysis been performed.

It was also felt that an analysis of the plasma lipids of birds would provide information of value in connection with another aspect of lipid metabolism, namely, the relation of plasma lipids to diet. The lipid content of plasma is in general somewhat, and often markedly, lower in herbivorous animals such as rabbits (4), guinea pigs (6), goats (1), cows (8), and steers (10) than in omnivorous animals such as man (3) and dogs (7). Bloor (2) has found that the blood lipid levels of rabbits may be altered by changes in diet to a greater extent than those of dogs.

Methods

The cockerels used were obtained from a poultry farm in the spring of the year, when they were one pound in weight. They were housed in a large

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room, with complete liberty of movement, and were fed on Purina Chick Growing Mash and whole wheat grain with crushed oyster shell and water ad libitum. They were killed in the early autumn, and weighed between 1200 and 1600 gm. each. At this time they appeared to be in excellent condition, crowed lustily, and had well developed combs. An autopsy was performed immediately after drawing blood for lipid analysis, and there were no pathological findings. Potassium oxalate was used as an anticoagulant for the blood, which was collected from the neck vessels. The blood was immediately centrifuged and the plasma extracted and analysed by oxidative micromethods (5).

It is an accepted and unfortunate fact that "blood lipid" values vary according to the technique employed, especially colorimetric estimations of cholesterol versus the oxidative method. A discussion of some factors that may affect lipid estimations has recently been made (5). Not only is there a variation from technique to technique, but occasionally two or more analysts may fail to obtain the same results with the same technique. After sufficient experience however, a qualified analyst may repeat his own results within a coefficient of variation of less than 5%. Comparative studies of blood lipids by the same analyst with the same technique yield data which do not require the necessity of estimating the effect of differences in technique.

Results

A statistical summary of the results is given in Table I. Most of the values were notably similar to those in normal human blood, analysed by these same methods, and as notably different from the lipid values of rabbit and guinea

TABLE I

THE LIPID COMPOSITION OF BLOOD PLASMA IN 22 YOUNG WHITE LEGHORN COCKERELS

(Results are expressed in mg. per 100 ml.)

Value	Total lipid	Composition of total lipid					
		Neutral fat	Total fatty acids	Cholesterol			Phospho-lipid
				Total	Ester	Free	
Minimum	368	115	248	65	31	19	90
Maximum	704	374	503	154	106	54	240
Mean	520	225	361	100	66	34	155
Standard deviation	85	77	74	23	19	9	34
Coefficient of variation	16	34	20	23	29	26	22

pig blood. The various cholesterol fractions and phospholipid were somewhat lower in value than those in man and the neutral fat somewhat higher. All of the values were considerably higher than previously found by the author in the blood plasma of rabbits and guinea pigs (4, 6). We have to the present determined lipid values in the blood plasma of four different species, and the

various means of these analyses have been collected in Table II. It is hoped that an opportunity may later be provided to extend this comparative study.

TABLE II

A COMPARISON OF THE MEAN VALUES FOR PLASMA LIPIDS IN HUMAN, COCKEREL, RABBIT, AND GUINEA PIG BLOOD BY THE AUTHORS' OXIDATIVE MICROMETHOD

(Results are expressed in mg. per 100 ml.)

Species	Total lipid	Composition of total lipid					
		Neutral fat	Total fatty acids	Cholesterol			Phospho-lipid
				Total	Ester	Free	
Human (3)	530	142	316	152	106	46	165
Cockerel	520	225	361	100	66	34	155
Rabbit (4)	243	105	169	45	23	22	78
Guinea pig (6)	169	73	116	32	21	11	51

In order to show the relation of values of the different lipid fractions one to the other, the results for cockerel plasma were divided into groups of increasing lipid values. Moving averages within these groups were then calculated by averaging the values in groups with total lipid values between

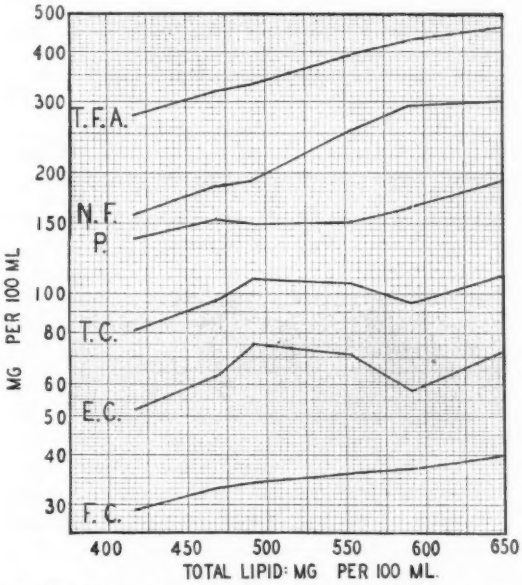

FIG. 1. *Arithlog plotting of changes in the moving averages of values for lipids in the blood plasma of healthy young cockerels with changes in the moving averages for total lipid.* T.F.A.—*total fatty acids;* N.F.—*neutral fat;* P.—*phospholipid;* T.C.—*total cholesterol;* E.C.—*ester cholesterol;* F.C.—*free cholesterol.*

350 and 450 mg. per 100 ml., between 400 and 500 mg., between 450 and 550 mg., and so on. The resulting moving means were then plotted on arithlog paper and this chart is given in Fig. 1. It may be seen again that in general when there is more of one lipid present in plasma there is correspondingly more of another. As in rabbit plasma (4), the chief fluctuations occurred in the values for ester cholesterol, which erratically increased with increase in total lipid. It should be emphasized again that these interrelations have been demonstrated in *normal* animals. In conditions accompanied by a lipopenia or lipemia, such correlation may not—and in instances where it has been studied does not (3)—exist.

The results indicate further that plasma lipids in normal herbivorous animals need not necessarily be lower than those in omnivorous animals.

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THE CARBOHYDRATE METABOLISM OF DEVELOPING SALMON EGGS[1]

By F. R. Hayes[2] and Andrew Hollett[3]

Abstract

The glycogen and glucose contents of salmon eggs, detached embryos, yolk sacs, and livers have been estimated periodically from fertilization to the end of yolk absorption. Glycogen is absent at the beginning, appears midway between fertilization and hatching, and later increases steadily in concentration, reaching a maximum of 0.53 mg. per 130 mg. egg. Glycogen storage by the liver begins suddenly some time after hatching, before which the muscles serve as a storage place. No glycogen ever appears in the yolk. The initial quantity of glucose is 0.064 mg. per egg. The concentration increases with development, is interrupted at hatching, and then continues to rise, reaching a maximum value of 0.29 mg. per egg. The glucose is distributed throughout all parts of the system, and its fluctuations parallel those of the water content, leading to the suggestion that the glucose concentration per unit of water is constant throughout development.

This investigation was carried out to determine the carbohydrate content of salmon eggs and larvae up to the end of the yolk-sac stage, in order to obtain information regarding the metabolism of carbohydrate and its place of storage. The free sugar content and the glycogen content were determined of: (i) the whole egg or larva (embryo plus yolk); (ii) the embryo only; (iii) the yolk only; (iv) the liver. Where the word "embryo" is used in this paper, it means "embryo dissected off the yolk sac".

Carbohydrate in eggs and animal tissues may be present in three forms, namely free glucose, free glycogen, and a carbohydrate complex forming part of a protein molecule. Of these we have estimated only the first two, so that when we speak of "total carbohydrate" we mean "total free carbohydrate". Of the third fraction, attached to protein, it has been generally assumed that it is absent or negligible in the yolk of chick eggs, and was in fact thought to be exclusively a part of the mucins and mucoids, which in their turn are thought to be essentially confined to the albumen. It is now known, however, to be present in all the fractions of albumen (21) and its presence in chick egg yolk has been reported at least once (6). The complex appears to be made up of four molecules of mannose, two molecules of glucosamine and an unidentified compound of nitrogen (17). (Neuberger (17) found that the carbohydrate could not be split off from the protein by any physical means such as recrystallization, ultra-filtration, denaturation with acid, or denaturation with heat, so that the method used by us would not yield any of it.) Nothing is known of the proteins inside a salmon egg. We are of the opinion that any combined carbohydrate present is not in sufficient quantity to account for the observed rise in free carbohydrate (Fig. 1), although we have no proof of this for the

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salmon. In the frog, however, only 10 to 15% of the carbohydrate is in the combined form (4), while in the chick the combined carbohydrate makes up one-third of the total, and nearly all of it must be in the albumen (13, 14). The only salmon egg protein which has been investigated is that of the shell or casing, in which Young and Inman (22) found that glucosamine made up 1.04% of the dry weight. This quantity would be negligible for embryonic use even if it were passed over to the embryo, a point on which there is no evidence.

Methods

Several thousand recently fertilized, and a smaller number of unfertilized, eggs of the Atlantic salmon, *Salmo salar*, were obtained from the Government Fish Hatchery at Bedford, N.S., at the time when the eggs were being fertilized in November of 1937 and 1938. The fertilized eggs were reared in a hatchery at the University at an average temperature of 7° C., and determinations of the glucose and glycogen content of eggs or larvae were made weekly. Carbohydrate analyses of detached yolks, embryos, and livers were also periodically made.

The sum of the carbohydrate present in the detached embryo and yolk was less than that obtained from an analysis of the whole larva. This was to be expected since the detachment of the yolk sac caused the rupture of all blood vessels connecting the embryo with the yolk, which necessarily must have resulted in a slight loss of blood. The error for glycogen was not great, but the glucose loss was sometimes 10 to 20%. However, analyses of whole larvae were usually made at the time that carbohydrate determinations of egg fractions were carried out, and Table I has been constructed from data of both kinds.

It was found that the wet weight of eggs varies from year to year; in 1937 the average weight of recently fertilized eggs was 116 mg.; in 1938 the weight was 149.75 mg. Hayes and Ross (8) encountered a like variation in weights. They therefore invented a standard fertilized egg of 130 mg. wet weight and adjusted their figures on this basis. A similar procedure was followed in this investigation.

In the results to follow, ages have been expressed as the number of days before or after hatching. Introduced by Allen (1), this method of expressing the age of the salmon eggs and larvae decreases the error caused by varying developmental rate from year to year due to temperature variations of the water in the aquarium. The date on which most eggs hatched was taken to be the zero day or central date of hatching.

The carbohydrates present were obtained by Soxhlet extraction with water carried out for 3 to 5 hr. Material to be extracted was first placed in boiling water to destroy any enzymes capable of converting glycogen to glucose (12, p. 185). The number of eggs or larvae used in a test varied from 20 to 40 depending on the carbohydrate content.

Because of the solubility of glycogen in water (7) it was possible to estimate both glycogen and glucose from a single sample. Each extract was divided

into two portions. On one the glucose was estimated directly; in the other the glycogen was converted to glucose by acid hydrolysis before the glucose determination. The glucose value of the second portion, minus that of the first portion, multiplied by 0.927 gave the glycogen concentration (16). The customary factor for conversion of glucose to glycogen is 0.927, although Kerly (9) recommended 0.957, while Kerr (10) found the factor in acid hydrolysis to be 0.938.

Sugar was estimated by the method of Patterson (18), whose procedure was slightly modified by the use of 2 ml. of aqueous extract instead of 0.2 ml. By performing the experiment with standard glucose solutions a series of values was obtained from which a graph similar to that given by Patterson was constructed. The method is basically that of Hagedorn and Jensen, from which it differs only in the manner of determining the amount of reduced ferricyanide.

The Carbohydrate Content of Eggs and Whole Larvae

Determinations were made from the day of fertilization until the yolk sac had completely disappeared, 92 days after hatching, The results are indicated in Fig. 1 and in Table I. Glucose plus glycogen equals total carbohydrate.

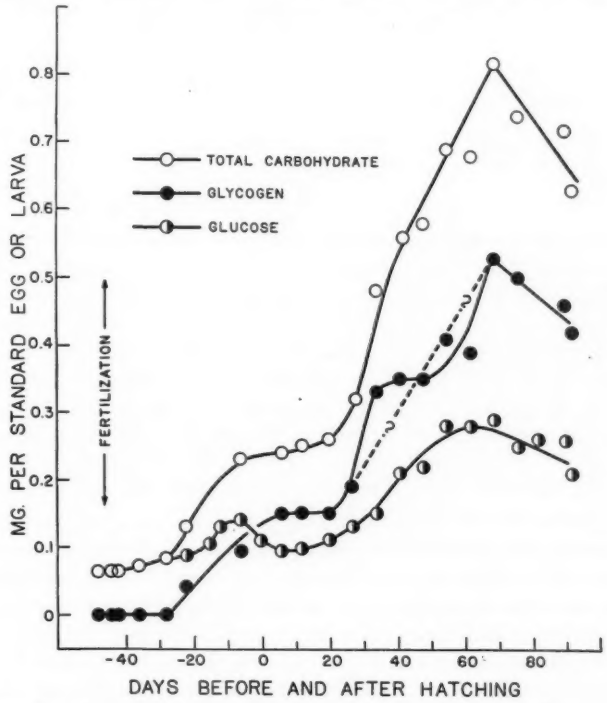

FIG. 1. *Distribution of carbohydrate in the whole egg or larva.*

TABLE I

RESULTS OF ANALYSES OF SALMON EGGS, LARVAE, AND CONSTITUENT PARTS OF THE SYSTEM

All figures are in milligrams per egg, assuming that an egg weighs 130 mg. in the early stages

1 Days before and after hatching	2 Yolk sac glucose	3 Embryo glucose	4 Total glucose	5 Embryo or total glycogen	6 Total carbohydrate	7 Liver glucose	8 Liver glycogen
Unfert. eggs			0.064	0.000	0.064		
−44			0.065	0.000	0.065		
−42			0.066	0.000	0.066		
−36			0.071	0.000	0.071		
−28			0.083	0.000	0.083		
−22			0.089	0.041	0.13		
−15			0.104				
−12	0.10	0.028	0.13				
− 6			0.14	0.094	0.23		
0	0.085	0.025	0.11				
6	0.062	0.033	0.095	0.15	0.24	0.004	0.01
12			0.099	0.15	0.25		
20	0.051	0.045	0.11	0.15	0.26		
27			0.13	0.19	0.32	0.0027	0.0073
34	0.053	0.084	0.15	0.33	0.48		
41			0.21	0.35	0.56	0.0028	0.04
48	0.07	0.13	0.22	0.35	0.58		
55			0.28	0.41	0.69		
62	0.10	0.14	0.28	0.39	0.68	0.0043	0.10
69			0.29	0.53	0.82		
76	0.083	0.14	0.25	0.50	0.74	0.0062	0.15
82			0.26				
90			0.26	0.46	0.72	0.009	0.22
92			0.21	0.42	0.63		
Yolk sac gone							

The carbohydrate content of eggs two days after fertilization was 0.065 mg. This was all in the form of free sugar; no glycogen was found until three weeks after fertilization. The amount of glucose in unfertilized eggs was estimated to be 0.064 mg. These determinations were made on four batches of eggs varying from 25 to 35 in number. The first two batches contained an average of 0.063 mg. of glucose, while an average of 0.064 mg. was present in the second two. Duplicate analyses were nearly always made of whole eggs. Since all glycogen in advanced stages was in embryos only, therefore the same was assumed to be true in the early stages when the embryo could not be dissected from the yolk sac. This assumption gives the early points in the embryonic glycogen curve as drawn (Fig. 3).

Except during the period of hatching, there is an increase in carbohydrate from the time of fertilization up to about 70 days after hatching when nearly all the yolk has been absorbed. The maximum concentration is 0.82 mg. per larva. There was a continued decrease in carbohydrate after this time.

A close parallel was observed between increase in glucose and in glycogen in the complete larval system, though the maximum concentration of glycogen is reached a few days after that of glucose.

From a week before hatching to a week after it there is a diminution in glucose. There is, however, no decrease in glycogen, although the curve flattens for three weeks after hatching, following which there is an uninterrupted synthesis of glycogen up to about 70 days, after which it declines.

It is notable that Hayes and Ross (8) found a drop in fat content of the embryo at hatching. Moreover Saunderson (19) found that cartilage formation in the salmon head ceased abruptly at the time of hatching. A comparison of the cartilage in the head and the carbohydrate in the larva is made in Fig. 4. It will be seen that the synthesis of carbohydrate ceases abruptly before hatching and is resumed about three weeks later. In cartilage formation also the curve flattens out for nearly three weeks, after which there is a rapid rise until it reaches a maximum. The similarity of the curves lends support to the suggestion that at hatching or birth there is a pause in the building of new structures, and in the synthesis of new materials. We do not mean to suggest by Fig. 4 that carbohydrate and cartilage have a causal relation to one another, but we do suggest that carbohydrate or fat are morphological entities in the embryo, subject to the same developmental laws as cartilage or any other tissue or organ.

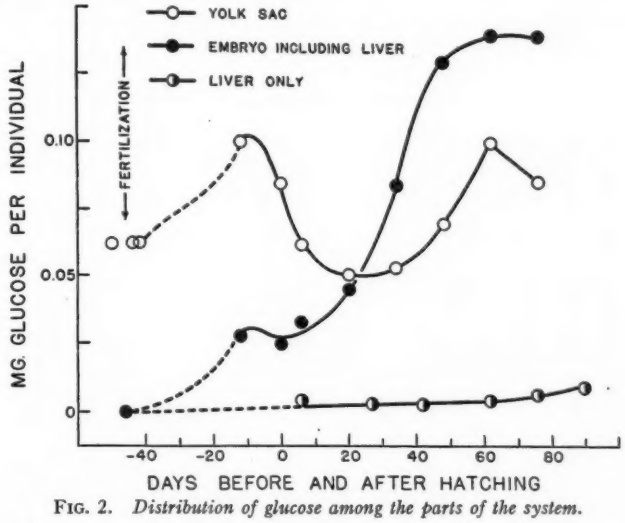

FIG. 2. *Distribution of glucose among the parts of the system.*

From a further examination of Fig. 1 it is evident that the mechanism for the building up of glucose is present from the beginning, since there is an increase in glucose a few days after fertilization. Analyses for glycogen, however, gave negative results until 24 days after fertilization. In duplicate tests a week before this no glycogen was found. This suggests that the mechanism for the synthesis of glycogen is not present in the egg soon after fertilization, but appears somewhat suddenly about three weeks before hatching.

Carbohydrate in Embryos

Determinations of the amount of glucose and glycogen present in the embryos (yolks detached but livers present) were made 12 days before hatching. This was the earliest time at which it was found possible to detach sufficient embryos to make an analysis. After that time the carbohydrate content of embryos was measured about every two weeks. It will be seen from Fig. 2 that glucose rises steadily, sloping off somewhat at hatching, following which it increases fairly rapidly and uniformly until some 60 days after hatching. A similar trend is noted for glycogen (Fig. 1) but the peak is reached 12 days later. It should be noted that "embryonic glycogen" is the same as "total larval glycogen", since all the glycogen is in the embryo.

We have been uncertain what value to give to the apparent flattening of the glycogen points between 30 and 50 days (Fig. 1), so we have drawn the curve both to show the flattening (solid line) and to suppress it (dotted line). In Fig. 3 the "embryo minus liver" curve is derived by subtraction of liver glycogen from the curve in Fig. 1, and in Fig. 3 the two glycogen possibilities are shown between 20 and 70 days.

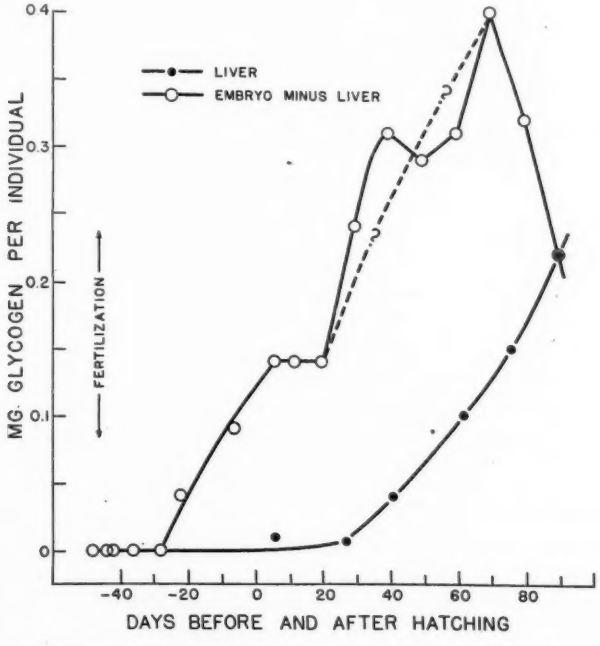

FIG. 3. *Distribution of glycogen between the liver and the rest of the embryo. The sum of these two curves appears in Fig. 1 as glycogen in the whole egg. Values for "embryo minus liver" have been obtained by reading from the Fig. 1 glycogen curve, and subtracting the liver curve. In the period from 20 to 70 days, the solid and dotted lines correspond respectively to the same region of the glycogen curve in Fig. 1.*

Detached Yolk Sacs

The carbohydrate in the yolk was estimated at the time that analyses were made of the embryos. The values are given in Fig. 2. The glucose in the yolk reaches a peak twice during larval development: about a week before hatching, and at 62 days when ordinarily the larvae would probably be feeding. As already stated, the earliest analyses of detached embryos and yolks were made 12 days before hatching. The curves in Fig. 2 to the left of −12 days have been drawn in dotted lines, their shape being based on the following evidence: at fertilization all the 0.65 mg. of egg glucose must have been in the yolk, since there was no embryo. Similarly, the embryonic glucose at fertilization was zero. Between fertilization and −12 days the sum of the embryonic and yolk curves must give the values in Table I, column 4.

When the last analyses were made the yolk was almost completely absorbed, and the sac could be detached only with great difficulty. At the time of the final analysis of larvae at 92 days the yolk sac had completely disappeared.

No glycogen was detected in the yolk sac except on two occasions, and then in such minute quantities—of the order of 0.01 mg.—that it might well be attributed to error. Therefore, all the glycogen present in the larval system was stored in the embryo.

Livers

Periodic estimations were made of the carbohydrate content of the liver. The results are shown in Figs. 2 and 3. Only minute quantities of reducing sugar were found. An examination of the graph indicates that the liver

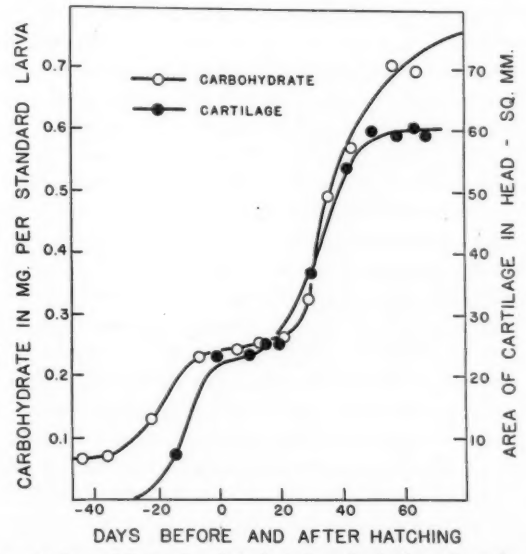

FIG. 4. *A comparison of the total carbohydrate in a developing salmon larva with the area of cartilage in the head. Cartilage values are from Saunderson (19).*

does not act as a storage place for glycogen until some 30 to 40 days after hatching, when this function is rather suddenly assumed. After this time the glycogen content rapidly increased until the last analysis was made 90 days after hatching, when 0.222 mg. were stored there. This rapid rise in glycogen will be discussed later.

Discussion

From Fig. 2 it will be observed that the embryonic and yolk glucose tend to rise and fall together. The simplest explanation of this is found in the suggestion that glucose in an embryo, as elsewhere, is not stored in any specific tissue, but passes freely through all walls and is distributed throughout the larval system as an aqueous solution equally concentrated in all parts. According to this interpretation the ratio of glucose between embryo and yolk at any time serves merely as an index of the relative quantities of water in each. It also follows that at any time, the embryo with 80 to 90% water will contain more glucose per gram wet weight than the yolk with 50 to 60% water.

Fig. 2 shows that the synthesis of glucose from some source (not glycogen) begins at the time of fertilization. It is presumably accomplished by means of enzymes within the embryonic cells and will therefore be proportional in intensity to the number of such cells. This synthesis of glucose never stops during development, although it is quite possible that its rate diminishes for a short time before hatching. Against this continual synthesis we have to set *(a)* the utilization of glucose as fuel, which undoubtedly goes on all the time although we cannot express it quantitatively, and *(b)* the removal of glucose as glycogen. The capacity for glycogen formation is not, like that of glucose, present from the beginning, but appears rather suddenly some 28 days before hatching. Histochemical studies showed that the place of storage was chiefly the muscles; those of the caudal and dorsal fins stained markedly with 0.1 *N* iodine solution. Probably the onset of glycogen storage corresponds to the time in embryonic development when muscle tissue becomes differentiated. Glycogen storage by the liver is discussed below, but it may be noted here that during the whole larval period muscle storage is much the more important part, an observation which points to the danger of interpreting embryonic glycogen as a demonstration of liver storage.

In Fig. 1 a rather constant time lag between glucose and glycogen fluctuations will be noticed. Thus glucose begins to diminish some eight days before hatching, and two weeks later the synthesis of glycogen stops. Six days after hatching glucose is synthesized again, and two weeks later the same thing happens to glycogen. At 60 days the glucose curve again turns down, and again 10 days later the glycogen curve follows. This last change happens in spite of the fact that the liver glycogen pursues its course unaltered. The suggestion comes very readily that we are dealing in the muscle cells (though not in the liver) with a reaction

$$\text{Glucose} \rightleftharpoons \text{Glycogen}$$

which may proceed in either direction depending on the relative concentrations of the two substances. The embryo is therefore taking any of the new glucose that is left over after metabolic needs are satisfied and converting it into muscle glycogen. In time of need this muscle glycogen is drawn on as a reserve. The final drops in all three curves after about 70 days are to be interpreted as the onset of starvation, for at this time the yolk is almost used up. By the end of our observations at 92 days there was no yolk sac visible at all.

It is interesting to find that Shaw (20) noted the disappearance of glycogen from the livers of chicks on the day of hatching, although glycogen was present in the liver the day before and two days after hatching.

One of the most striking things in these analyses is the sudden appearance of glycogen in the liver some 40 days after hatching, and its rapid rise after this time. Fig. 3 represents the glycogen in the liver and in the embryo minus liver. The curve for the embryo without liver rises steadily until the 68th day, and then declines sharply. This corresponds to the time when the storage of glycogen by the liver is taking place most rapidly. The yolk has not yet been completely absorbed, and there is still an increase in total embryonic glycogen.

The sudden glycogenic function of the liver has been noted by many investigators, and appears to be almost universal among the vertebrates. Lochhead and Cramer (11) found that though definite amounts of glycogen were present in foetal livers of rabbits, the percentage was very low up to the 25th day. This represented the time when the liver assumed its adult glycogenic function. They noted that this coincided with the beginning of the decrease in placental glycogen.

In studies on various embryos, Aron (2) showed that in the pig the liver functioned as a storage place for glycogen near the end of gestation. In the sheep, glycogen is found in appreciable quantities at the end of the fourth month of pregnancy, and in the guinea pig the glycogenic function is assumed more slowly but definitely in the last four days of gestation. He discussed the appearance of the islets of Langerhans out of the islets of Laguesse, and found a striking coincidence between the appearance of the islets of Langerhans in the pancreas and the deposition of large quantities of glycogen in the liver. Moreover, he endeavoured to show that the islets of Langerhans produce insulin, but those of Laguesse do not. From further investigations by histochemical methods Aron (3) found no glycogen in the liver before the appearance of the hind limb buds, and that removal of the pancreas in early stages prevented the appearance of glycogen in the liver. As a result of his research, Aron concluded that the beginning of this function of the liver varies from species to species but manifests itself at a fixed time in ontogeny. The present work supports his theory.

There is, of course, no pancreas in teleosts, but in the adults there are well-defined masses representing the islets of Langerhans. Whether the appearance of glycogen in the liver of the salmon is related to islet tissue must remain for

the present a matter of speculation, since no reference has been found to the time of its appearance.

There is a synthesis of carbohydrate from fertilization, uninterrupted except at the time of hatching, until the beginning of the final starvation period. We have examined the literature but can find no other case in which the embryo ends up with more carbohydrate than the egg had at the beginning. This unique property of the salmon egg is no doubt shared by other teleosts. We do not know what the source of this new carbohydrate is. Certainly the great period of carbohydrate synthesis after hatching corresponds to a sharp drop in fat (8), although, since the carbohydrate gain amounts to only one-tenth the fat loss, the two cannot be said to be quantitatively related. For the three weeks before hatching there is a synthesis of both fat and carbohydrate, although the absolute quantity of carbohydrate involved is quite small, of the order of 0.1 mg. per egg. This gain might be accounted for by the combined carbohydrate in protein molecules or by the protein itself.

A synthesis of glycogen (as distinct from total carbohydrate) is found in the chick as well as in the salmon (13). In the salmon the glycogen is all in the embryo. In the chick, however, Needham divided the system into "embryo" and "remainder of the egg". The latter part included not only the yolk and albumen, but the allantois, amnion, and the coelom with its two walls each composed of two germ layers. It is not surprising therefore that the newly formed glycogen was found in both parts. Needham does not suggest, nor do we see any evidence for, the storage of glycogen in the yolk or albumen. Its storage as in the salmon is evidently cellular, the extra embryonic portion being laid down in the blastoderm. In our method of separating the yolk sac from the salmon embryo, the division was made in the coelom, so that the somatic mesoderm and ectoderm were included with the embryo, the splanchnic mesoderm and endoderm with the yolk sac. The quantity of cellular material which went with the yolk sac was a very small proportion of the whole embryo. The glycogenic function of the extra embryonic membranes of the chick is probably something new which has arisen with the membranes themselves.

The view has been expressed that glucose traverses cell walls and is found everywhere in the salmon system, including the yolk. Glycogen on the other hand is evidently confined to cells. If the enzymes necessary to synthesize glycogen act only in cells, it is, to say the least, probable that the enzymes which promote the production of glucose from glycogen also act in cells. They may very well be the same enzymes. With this distinction between glucose and glycogen in mind, we may examine Table II, which shows the stores of carbohydrate with which different eggs start out. So far as investigated, eggs of birds, reptiles, and fishes, have their initial store as glucose, with little or no glycogen. Amphibia and invertebrates on the contrary have stores of glycogen with little or no glucose. The only exception is the trout egg, in which Fauré-Fremiet and Garrault report 0.34% glycogen. About the same time as the trout work, however, Fauré-Fremiet and his collaborators were

TABLE II

THE CONCENTRATIONS OF GLUCOSE AND GLYCOGEN IN VARIOUS EGGS AT THE TIME OF FERTILIZATION

All figures are in per cent wet weight

Animal	Glucose	Glycogen	Author
Chick, whole egg	0.20	0.003	Various authors
Chick white	0.50	0.003	Sakuragi
Chick yolk	0.33	0.002	Sakuragi
Tortoise	0.14		Diamare
Frog		2.5	Various authors
Herring	0.50		Steudel and Osato
King salmon	0.096		Greene
Atlantic salmon	0.050	0.000	This paper
Trout		0.34	Fauré-Fremiet and Garrault
Starfish		0.020	Dalcq
Sea urchin *(Echinus)*		1.3	Moore, Whitley, and Adams
Sea urchin *(Strongylocentrotus)*	0.08	1.28	Ephrussi and Rapkine (5)
Silk worm		1.5	Various authors
Bee		2.5	Straus
Sabellaria (Polychaet worm)		1.3	Fauré-Fremiet

NOTE.—*Chick figures are from Needham (15, Vol. 1, p. 278), other figures are from Needham (15, Vol. 1, p. 356), except Strongylocentrotus (for which see (5)) and our salmon figures.*

reporting glycogen concentrations in frog eggs much greater than those found by others—sometimes four or five times as high as the average of other workers. For this reason we are inclined, pending confirmation, to disregard the trout figure.

If we look for an underlying morphological explanation for Table II, we find that the "glycogen" eggs are those in which the whole egg divides into cells that participate in development. In the frog, for instance, though the cells in early development differ markedly as to size, each cell will contain a store of glycogen. One does not think of the embryo as taking material from the yolk, but rather of the yolk cells continuing to divide and changing gradually into differentiated embryonic cells. The stores are therefore divided from the outset.* There is a time rather early in development when most of the activity is in small cells with limited stores, and conversely, most of the stores are in the relatively inactive yolk cells. It is during this time that the active cells, with the glycogen store (as we think) locked away from them, use up most of the available combined carbohydrate (4). Later the problem solves itself by cell division, which breaks up the storage cells and distributes them; and by the acquisition with continued differentiation, of glycogen-splitting enzymes in the former yolk cells. The same argument applies with even fewer provisos to the eggs of echinoderms, while in the insects the nucleus continues to divide without cell walls until the several hundred nuclei migrate to the surface and cell walls are formed. All the egg material

* *The division of stores is clearly illustrated by unpublished microphotographs of Amblystoma larvae which Dr. Herbert Coar has kindly shown us. Here the muscle cells, after the differentiation of fibres and cross striations, still contain numerous yolk platelets.*

therefore participates in early development. The polychaetes have total spiral cleavage, so that again there is nothing corresponding to a yolk sac.

Turning to the "glucose" eggs of birds, reptiles and teleosts, it is found that the embryonic stores are locked away in the large, non-cellular yolk sac. As the small embryo uses up glucose it is replaced by simple diffusion from the yolk, a mechanism that would not serve if glycogen made up the carbohydrate store. The glucose taken by the embryo is constantly replaced and augmented, although we do not know where or how. Now if we were to take a salmon egg, which is about half water at the beginning, and by the addition of water to make it two and one-half times as heavy as it was before, it is obvious that there would be four times as much water as before, and that if the glucose per egg remained the same, the glucose tension would drop to one-quarter its former value. If at the same time as the water, we added enough glucose to make a fourfold increase per egg, then the glucose tension would remain unchanged. In a salmon larva at the end of the yolk sac period there actually is four times as much water, and as Table I, column 4 shows, four times as much glucose, as well. We are inclined to conclude that the glucose tension throughout development remains the same, and that, as already mentioned, when glucose tends to exceed the fixed level glycogen is formed, and vice versa. It will be possible to formulate this theory quantitatively when we have a good set of wet and dry weights. At first sight the chick appears to develop in the opposite manner, since there is a diminution in glucose per egg during development, but it must be remembered that the chick gets drier, not wetter as it develops, and moreover the part played by the amniotic fluid with respect to glucose is not known. It is quite possible that the glucose tension in terms of embryonic chick water is constant throughout development.

In conclusion mention may be made of the rate at which new carbohydrate is formed in the salmon. We cannot do this quantitatively, but an inspection of Fig. 1 shows that the curve for total carbohydrate is steepest in the periods −28 to −6 and 24 to 45 days. These probably correspond to the periods of maximum synthesis per unit wet or dry weight of embryo. The first of these fits very well with the table of anticipated energy sources previously published (8, p. 372). The second maximum, however, is something new and we do not at the moment see that it has any correspondence in chick embryology.

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PARASITES OF FRESHWATER FISH

II. PARASITISM OF SPECKLED AND LAKE TROUT AND THE FISH FOUND ASSOCIATED WITH THEM IN LAKE COMMANDANT, QUE.[1]

By L. L. Lyster[2]

Abstract

Parasitism of two game fish, *Salvelinus fontinalis* and *Cristivomer naymaycush* and of two other fish, *Perca flavescens* and *Catostomus commersonii* is discussed. *Glaridacris intermedius* sp. nov. (Cestoda), *Rhabdochona laurentiana* sp. nov. (Nematoda) and *Raphidascaris alius* sp. nov. (Nematoda) are described.

In presenting further information on parasitism in freshwater fish in the Province of Quebec it is hoped that a contribution will be made not only to distribution records, but also to the interrelation of parasitic forms. The first paper of this series (13) dealt with general trematode parasites. Collecting was done over an extended period and in a considerable area, and although several trematodes were found in two or more hosts, it was impossible to judge what part any fish might play in maintaining an infection in another. The present paper, on the other hand, is based on material collected in limited and known waters and within a short period of time. This material was collected during the months of September and October, 1938, in Cameron's Bay, a southerly projection of Lake Commandant.

Four hosts are considered, *Salvelinus fontinalis*, *Perca flavescens*, *Catostomus commersonii*, and *Cristivomer naymaycush*. Of these, the first three, speckled trout, yellow perch, and common sucker, were invariably found together. The fourth, lake trout, was never found in association with the other fish, but was taken in the deeper parts of the same waters; it was included in the survey in the hope that its parasite records might indicate whether identical parasitism was more apt to occur among totally unrelated fish of similar habitat, or related fish living under different conditions.

Trematodes

Two species of *Crepidostomum* were collected, one from perch and one from speckled trout. In addition a trematode reported as a new species from perch in the first paper of this series was found in small numbers in the perch and in considerable quantity in the trout. Both suckers and lake trout were free of trematodes. Metacercariae of a member of the genus *Apophallus* were present in large numbers in the skin of speckled trout, and metacercariae of a strigeid were noticed in small numbers in three of the perch. These immature forms will be discussed later.

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Crepidostomum isostomum Hopkins, 1931

HOST: *Perca flavescens*

Material: three specimens

These hexapapillate forms have features that suggest identification as either *Crepidostomum isostomum* Hopkins 1931, or *C. canadense* Hopkins, 1931. Measurements of major structures are very similar to those of *C. isostomum*, but are consistently greater, and uniformly twice those for *C. canadense*, except that of width. The ratio, length of pharynx to diameter of oral sucker, the shape of the ovary and its position in relation to the ventral sucker are different from *C. isostomum* but similar to *C. canadense*. The post-testicular condition of the vitellaria is unlike that described for *C. canadense* but like *C. isostomum*. With these exceptions, all considered variable, the present material could be assigned to either of the species. *C. isostomum* and *C. canadense* are thought by their author to be closely related (8), and since this material seems to possess characters of both species, *C. canadense* has been listed as a synonym of *C. isostomum*.

Three of these trematodes were recovered from a single yellow perch from the lake. They were long, narrow forms 2.5 by 0.575 mm., widest and deepest in the ovarian region, tapering to a point terminally and narrowing to a neck posterior to the prominent hexapapillate oral sucker. Oral sucker and acetabulum uniformly round, 0.25 mm. in diameter. Reduced prepharynx. Pharynx slightly longer than broad, 0.074 by 0.098 mm. Oesophagus long, opening in caeca dorsal to acetabulum. Caeca terminating behind testes. Cirrus sac a short wide structure curving slightly dorsally but only slightly exceeding or not quite reaching anterior margin of acetabulum; 0.25 mm. in greatest measurement, opening into common genital pore anterior to intestinal bifurcation. Ovary lateral, globular, removed posteriorly from acetabulum by about its own diameter, 0.24 mm. Globular seminal vesicle contiguous with posterior margin of acetabulum to dorsal of ovary, slightly larger than that organ. Testes irregularly shaped, margin entire, medial, slightly oblique and posterior to ovary and receptacle, about 0.28 mm. in diameter; considerably removed from posterior end of body, close together but not contiguous. Vitellaria extend from cirrus sac to posterior end of body in lateral medial area, few follicles dorsally and meeting posteriorly but not overlapping behind testes. Uterus with few small eggs, convoluted in region of ovary and looping to anterior margin of anterior testis.

Crepidostomum cooperi Hopkins, 1931

HOST: *Salvelinus fontinalis*

Material: 50 specimens

The anterior extent of the vitellaria, the relative size of the pharynx and suckers, and the posterior position of the crural furcation are confusing details in these forms, as well as the comparatively large size of the eggs. The body size, the vitellaria, and general organization are characteristic of *C. cooperi*,

particularly the variant described as *C. fausti* by Hunninen and Hunter (10). The absolute measurements of the sucker and egg are suggestive of *C. farionis*, but, in proportion to body size, they are larger. The fact that the body is recurved and somewhat contracted (but not shrunken) anterior to the acetabulum, would perhaps explain the rather posterior position of the crural furcation and the more anterior genital opening than is typical, as well as the extensive vitellaria in this region. This condition and the extreme variability of the species will not allow for a diagnostic importance for these atypical details, and the form has, therefore, been listed as *C. cooperi* Hopkins, 1931.

They are compact, broad, thick forms, 0.70 to 1.49 by 0.25 to 0.56 mm., body widest at the level of the oral sucker or at the acetabulum, or sides of body almost parallel and the ends bluntly rounded, thickest at the posterior margin of the acetabulum, and usually ventrally curved. Oral sucker subterminal 0.11 to 0.21 by 0.14 to 0.25 mm. with six non-prominent papillae rarely extending past limits of the body. Pharynx globular, about 0.08 to 0.015 mm. in diameter, dorsal and posterior to oral sucker, prepharynx, and pharyngeal vestibule. Oesophagus short, bifurcation at anterior margin or slightly in front of it. Crura extending well posterior to testes. Genital pore ventral to intestinal furcation. Cirrus sac long, slender, widest posteriorly passing dorsally to acetabulum to its posterior margin or slightly beyond, never filled by seminal vesicle. Acetabulum larger than oral sucker, 0.14 to 0.28 by 0.17 to 0.35 mm., greatest dimension laterally, medial, well anterior, and often almost in contact with oral sucker. Ovary globular, slightly lateral, dorsal of posterior margin of acetabulum, or slightly more posterior, 0.7 to 0.14 mm. Seminal receptacle varying in shape and size, and medial or submedial, not far behind ovary. Testes two, tandem, and globular or slightly wider than long, margins entire, 0.08 to 0.17 by 0.15 by 0.18 mm. removed by at least one diameter, and usually more, from terminal area of body. Uterus with many large intra-uterine eggs about 56 by 84μ extends as far as midpart of posterior testis. Vitellaria of large closely packed follicles filling all available space from anterior to posterior limits of body but somewhat limited dorsally. Excretory bladder obscured by these structures.

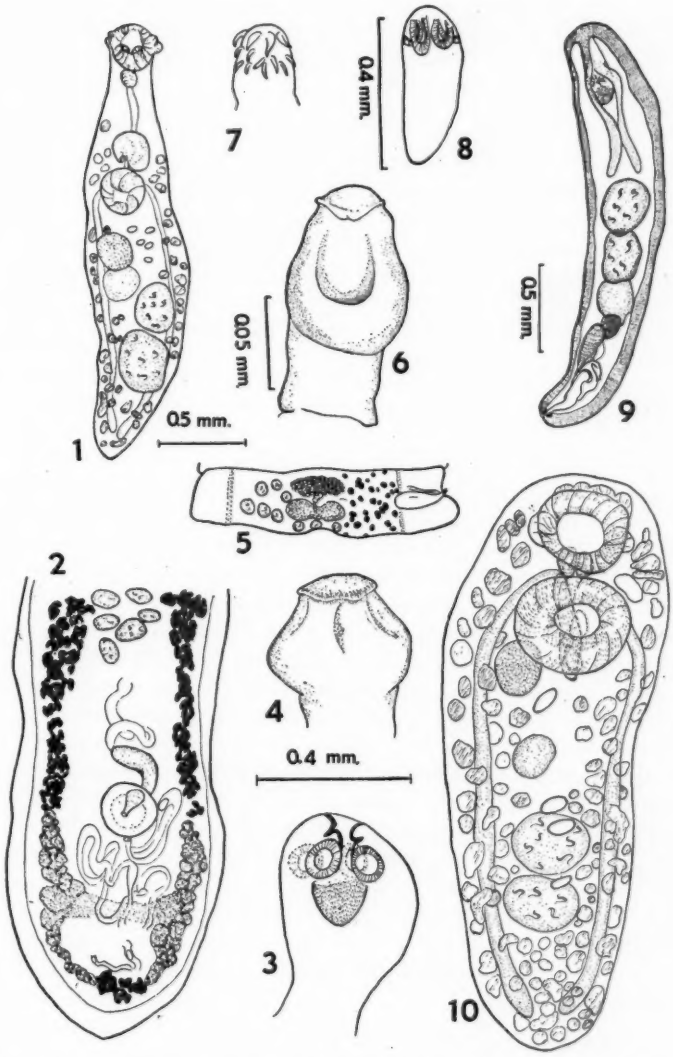
This species has already been reported from this host in Quebec by Richardson (15) under the synonymous name, *C. fausti* Hunninen and Hunter, 1933.

Ptychogonimus fontanus Lyster, 1939

HOSTS: *Perca flavescens, Salvelinus fontinalis*

Material: 25 specimens

This species was established in the first paper of this series from limited material collected from a perch (13). Many specimens were since collected from *Salvelinus fontinalis*, in the stomach and upper digestive tract. Little can be added to the original description of the species. In the present material it is noted that an oesophageal structure is present as a short, weak, saccate body, opening dorsally from the pharynx, and leading laterally and



FIGS. 1–10. *Trematodes, Cestodes, and Acanthocephala.* 1. *Crepidostomum isostomum from Perca flavescens.* 2. *Glaridacris intermedius sp. nov. posterior end, from Catostomus commersonii.* 3. *Ichthyotaenid from S. fontinalis.* 4. *Glaridacris intermedius sp. nov. anterior end, from C. commersonii.* 5. *Eubothrium salvelini mature proglottid from S. fontinalis.* 6. *Eubothrium salvelini scolex from S. fontinalis.* 7. *Neoechinorhynchus cylindratus—proboscis from S. fontinalis.* 8. *Ichthyotaenid from Cristivomer naymaycush.* 9. *Neoechinorhynchus cylindratus, entire worm from S. fontinalis.* 10. *Crepidostomum cooperi from S. fontinalis.*

anteriorly into the crura, a condition similar to that noticed in the related genus *Hirudinella* (4). The crura are naturally convoluted and much longer than the body cavity. This is uniform in all specimens and not due to contractions as previously suggested.

Cestodes

Polyzootic cestodes were limited to the trout. Adults of a single species and immature forms of this genus and another were found in *Salvelinus*. Similar immature cestodes were taken from the lake trout. Monozootic cestodes of a hitherto undescribed species were recovered from the common sucker. Perch carried no cestode infections. By far the heaviest parasitism noted was that of the polyzootic cestodes in speckled trout. All specimens of this fish carried some of the parasites and most had large numbers.

Eubothrium salvelini (Schrank, 1790)

Host: *Salvelinus fontinalis*

Material: 30 specimens

Presence of non-armed and non-specialized pseudobothridia, terminal disc, lateral genital opening, and saccate uterus show they belong to the genus *Eubothrium*. The shape of the scolex, length of the cirrus sac, and organization of vitellaria refer them to the species *E. salvelini* (Schrank, 1790), following the system of classification of Joyeux and Baer (12).

Scolex longer than wide, widest behind base of prominent pseudobothridia, and bearing bilobed terminal disc. Proglottids wider than long, about 1.68 by 0.7 mm. or greater, and considerably greater when compressed, always wider than scolex and often secondarily divided by transverse fissures. Genital opening lateral, alternating only rarely. Ovary bilobed, posterior central uterus saccate, anterior, central. Testes numerous, more or less spherical, about 0.07 mm. in diameter, occupying lateral intermuscular field and contiguous in neighbouring segments. Cirrus sac about 0.07 mm. at widest point in region of longitudinal nerve, about 0.6 mm. long. Cirrus broad, leaf-like when extruded, with minute spines. Vitellarian follicles small, scattered in central lateral fields. Vagina opening posterior but close to male genital openings.

Glaridacris intermedius sp. nov.

Host: *Catostomus commersonii*

Material: 10 adults

Four species have been listed by Hunter (11) in this genus of monozootic cestodes. Of these, two species, *G. hexacotyle* Linton, 1887 and *G. catostomi* Cooper, 1920, do not possess a terminal disc, while two species, *G. confusus* Hunter, 1929 and *G. laruei* Lamont, 1921, like the present form, have this structure in common, as well as non-operculate eggs and the absence of a genital atrium. Like *G. confusus*, this form has a granular ovary, not distinctly separate from the vitellaria, similar body shape and testes in two

lateral rows. Like *G. laruei* it has 70 testes and the vitellaria tend to enclose them. Measurements, egg size, etc., are much alike in *G. confusus*, *G. laruei* and the present form.

The significance of these similarities and differences is obscure at this time, and may indicate synonymities. The present form is apparently closely related to *G. confusus* and *G. laruei*, but cannot be referred to either. It must, therefore, be assigned to a new species which has been designated *G. intermedius* sp. nov.

The adults range in size from 3.5 to 9.0 mm. in length. They are widest at the level of the cirrus sac, 0.5 to 0.84 mm., and taper terminally to a rounded point, anteriorly to the region of the origin of the vitellaria, 0.4 to 0.55 mm. wide, which follows the narrower neck. The scolex is well differentiated and short, rounded at the junction with the neck, and squared anteriorly; it has three pairs of loculli and a terminal disc. All organs are entirely medullary. Vitellaria originate about 0.35 to 0.84 mm. behind the base of the scolex, extending as far as the ovary and with post-ovarian follicles. The testes are about 70 in number, arranged in two more or less parallel rows from a point slightly behind the origin of the vitellaria to about 0.30 mm. anterior to the cirrus sac; they are irregularly oval in shape, 0.07 by 0.14 to 0.07 by 0.084 mm. greatest diameter transversely. The vitellaria occur chiefly in the lateral areas, but scattered follicles are dorsal to the testes. The vas deferens is highly convoluted and enters a short, curved seminal vesicle dorsal and anterior to the cirrus sac. Cirrus sac small, circular, 0.084 to 0.085 mm. in diameter. Common genital atrium absent. H-shaped ovary varies in size, but wings usually about 0.5 to 0.7 mm. long; lateral branches relatively narrow, medial branch also limited. This organ is granular, finely lobate, similar in appearance to and in contact with lateral and terminal vitellaria. Uterus long, convolute, reaching to posterior extremity of body, and anteriorly to the cirrus sac; vagina medial, ventral. Seminal vesicle not obvious. Female system opens close to posterior edge of cirrus sac. Eggs non-operculate, about 42 by 28μ.

Larval Cestodes

Larval cestodes of two recognizable types were taken from both species of trout. Plerocercoid forms of *Eubothrium* were found in *Cristivomer naymaycush*. These consisted of a scolex and a short undifferentiated body. It is not possible to suggest the species to which these might belong, or to offer any details of their organization. The rather short scolex and deep pseudobothridia might be associated with *E. salvelini*. In other larval forms from this host four muscular suckers were present. These specimens seemed of two types. One consisted of a simple, saccate structure with four incomplete suckers alone distinguishable. In the other a fifth glandular structure was present; this type was also found in the speckled trout. These probably are referable to the family Ichthyotaeniidae Ariola, 1899 (Syn. Proteocephalidae Larue, 1911) as discussed by Joyeux and Baer (12).

ACANTHOCEPHALA

Acanthocephalids were present only in speckled trout and suckers. Light infections in each were caused by the same species.

Neoechinorhynchus cylindratus Van Cleave, 1913

HOSTS: *Salvelinus fontinalis*

Catostomus commersonii

Material: mature males and females were found in considerable numbers in the trout; one of each sex was taken from the sucker.

They were short, straight or slightly curved. Males were 3.0 to 4.9 by 0.6 to 0.82 mm., females about 5.1 by 0.82 mm. (longitudinal measurements exclusive of proboscis), broadest just posterior to the lemnisci. Proboscis longer than broad, about 0.53 by 0.12 mm., armed with three rows of six recurved hooks each. The anterior hooks were 0.4 to 0.45 mm. long, those of the two posterior rows were 0.21 to 0.03 mm. long. The hooks alternate in adjacent rows and are without collars or ornaments.

Embryos numerous and large, 0.07 by 0.10 to 0.12 mm. Lemnisci reach posteriorly almost to testis or almost half of body length. Brain located at base of proboscis receptacle. Testes closely applied, tandem, shortened cylinders, equal in size, 0.34 by 0.37 mm. Cement gland, a single body close behind testes, opening into cement reservoir. Small copulatory bursa present in males though often folded and inconspicuous. Genital openings terminal in both sexes.

NEMATODES

Nematodes are represented in the collection by two species from the speckled trout and one from the sucker. Material from the latter host was adequate, but that from the trout included few specimens and those of a single sex. Identification is, therefore, only as accurate as is possible with limited material.

Lake trout and perch were nematode-free.

Cystidicloides harwoodi (Chandler, 1931)

HOST: *Salvelinus fontinalis*

Material: one female; two immature males (uncertain)

This species was described as *Cystidicola harwoodi* from speckled trout by Chandler (2) and as *Metabronema canadense* by Skinker (17) from the same host. The latter author later placed it with *C. fischeri* in her new genus *Cystidicloides*, based on the nature of the post-cloacal papillae (18). This has been accepted by Van Cleave and Mueller (21), who reported it from *Salmo fario*.

The following description is based on the single female specimen.

Length 9.0 mm., greatest width 0.3 mm. Lips two, small and inconspicuous with slight chitinization at anterior corners, each bearing two papillae. Mouth simple, leading directly into tubular vestibule. Oesophagus in two distinct divisions; first part convoluted, occupying about 0.8 mm., second part 2.6 mm. long, emptying into simple intestine through a terminal sphincter. Divergent ovaries originate 0.15 mm. behind the oesophagus and just anterior to the anus; they are very convoluted. Bicornate uterus enters vagina just posterior to vulva, posterior uterine branch passes forward past this position to join vagina more or less parallel with anterior branch. At the level of the vulva the body diameter is sharply reduced by 0.112 mm. and on this projection the vulva opening is placed. Intra-uterine eggs 27 by 57μ; no terminal filaments noted. End of body bluntly rounded 0.116 mm. posterior to anus.

Two immature male forms slightly under 7.0 mm. in length and 0.14 mm. wide at the base of the oesophagus may belong to this species. The relationship is, however, not definite. The mouth and alimentary canal are essentially as in the female. The genital organs are poorly differentiated but a testis originates a short distance behind the oesophagus. The two spicules are dissimilar. The right member is short and wide, about 0.16 mm. long, pointed terminally but widening quickly to make the sides almost parallel; it is concave during the first three-quarters of its length and constricted just before the base. The left spicule is about 0.47 mm. long and narrow, with a widened base and a narrow wing rising at about the limit of the first quarter. Stalked, *not double* papillae present, four pre-cloacal and four post-cloacal. About 0.12 mm. behind the cloaca the body terminates in a blunt point. Conspicuous caudal alae extend about 0.028 mm. beyond the body margin.

Raphidascaris alius sp. nov.

HOST: *Salvelinus fontinalis*

Material: two males

The genus *Raphidascaris* is poorly organized and some of the descriptions of its species are inadequate, if not invalid. Several species have been reported from Europe and Asia and five North American species are already known. The present form cannot be assigned to any of these species. It differs widely in relative size of structures from *R. cayugensis* Wigdor, 1918 (23), *R. brachyurus* Ward and Magath, 1917 (22), and *R. anchoviellae* Chandler, 1935 (3) and, in the possession of similar alae and spicules, from *R. canadensis* Smedley, 1923 (19). It most closely resembles the trout species *R. laurentianus* Richardson, 1937 (16) in size and conformation, but the presence of lateral alae is a significant difference; Richardson's description of the species makes no mention of such structures. The same feature differentiates this form from *R. diadonus* Thwaite, 1927 (20); and the relative length of oesophagus and appendix are very different in *R. gigi* Fujita, 1928 as described by Yamaguti (24). Significant differences in size separate it from *R. acus* Bloch, 1779 (1) and *R. chirocentri* Yamaguti, 1935 (24).

Though the material is limited these differences necessitate the creation of a new species to accommodate it. This has been designated *Raphidascaris alius* sp. nov.

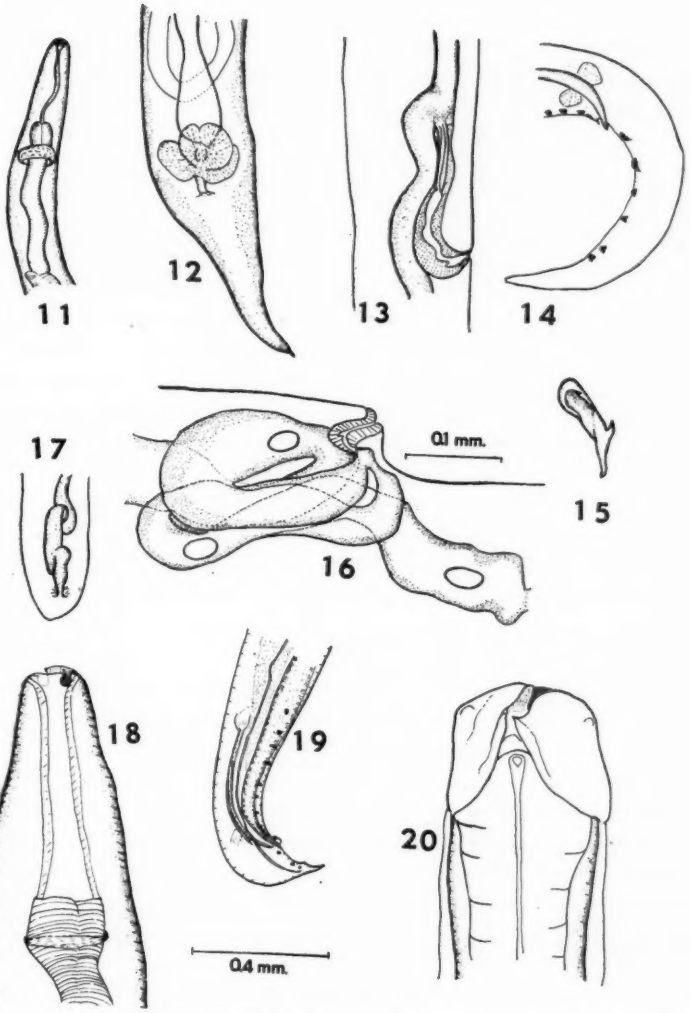
They are fairly large worms, 15.0 mm. long and 0.3 mm. wide at the base of the oesophagus and 0.4 mm. wide at the widest point. The head consists of three large lips bearing the usual papillae and conspicuous cuticular enlargements. The oesophagus is divided into a long anterior muscular portion 1.6 mm. long and a short sub-globular ventriculus 0.17 mm. long, which opens into an oesophageal appendix 1.3 mm. by 0.056 mm. and the simple intestine. The fore part of the body is curved ventrally and the tail is bent upon itself. The single testis rises just posterior to the end of the appendix, the length of the coils being greater than the width during most of its convoluted course. Just before entering the simple vas deferens, which is 0.7 mm. long, these coils are almost circular, giving a beaded appearance. Two equal and similar winged spicules are present, 0.63 mm. long and 0.028 mm. wide. They carry a terminal enlargement 0.084 by 0.112 mm. and taper to a fine point distally. The tail ends sharply 0.182 mm. behind the cloaca. On either side small pedunculate pre-cloacal papillae are present; anterior to these two or three small conspicuous elevated areas appear to mark the position of further more reduced papillae. Posterior to the cloaca three similar inconspicuous elevations probably represent the post-cloacal papillae. A conspicuous boss occupies the fore margin of the cloacal aperture. Equal lateral alae extend along the entire body length from a point just in front of the posterior extremity to the base of the lips. They are widest from the anterior limit to the region of the ventriculus.

Rhabdochona laurentiana sp. nov.

HOST: *Catostomus commersonii*

Material: 5 specimens of each sex; females non-ovigerous

This species and *R. cascadilla* Wigdor, 1918, the only other reported North American species, differ in several details, but notably in the length of the oesophagus. In Wigdor's (23) species, this structure is 1/25 the length of the body; in the present material it occupies one-quarter or more of the body length. Various differences are also apparent between it and the known European and Asiatic forms. Unlike *R. accuminata* Molin, 1860 (14) and *R. denudata* Dujardin, 1845 (5), it has a well marked pre-cloacal boss and greater numbers of papillae. Its spicules are dissimilar, while those of *R. salvelini* Fujiti, 1928, are similar (6). The terminal tail spine is not found in *R. gambiana* Gendre, 1922 (7). This form lacks the lateral teeth of *R. gymnocranii* Yamaguti, 1935, but possesses vestibular rods unlike *R. girellae* Yamaguti, 1935 (24). The extensive vestibule of *R. macrolainia* Gendre, 1922 (7), and the terminal spicular barbs of *R. zaconi* Yamaguti, 1935 (24), serve as points of differentiation, as does the position of the vulva opening in *R. amago* of this author.



FIGS. 11–20. *Nematodes.* 11. *Rhabdochona laurentiana sp. nov. anterior region female, from C. commersonii.* 12. *R. laurentiana sp. nov. posterior region female, from C. commersonii.* 13. *R. laurentiana sp. nov. vulvar region, female from C. commersonii.* 14. *R. laurentiana sp. nov. posterior end of male showing left spicule and post-cloacal papillae, from C. commersonii.* 15. *R. laurentiana sp. nov. right spicule, from C. commersonii.* 16. *Cystidicloides harwoodi, vulvar region, female, from S. fontinalis.* 17. *C. harwoodi, posterior end of female, from S. fontinalis.* 18. *C. harwoodi, anterior end of female from S. fontinalis* 19. *Raphidascaris alius sp. nov. posterior end of male from S. fontinalis.* 20. *R. alius sp. nov. anterior end of male from S. fontinalis.*

This form is, therefore, described as a new species, *Rhabdochona laurentiana*, the first member of the genus from *Catostomus commersonii* and the first from any Canadian host.

They are cylindrical forms, attenuated anteriorly, in which the cuticle is plain and unstriated throughout. The females are about 8.3 by 0.15 mm. with a long tail terminating in a distinct chitinized spine. The males are about 5.04 by 0.13 mm. with a long tail, but no specialized point. The lips and mouth are simple. The infundibuliform vestibule is supported by longitudinal rods which are free and pointed distally; it is about 0.08 mm. long. The oesophagus is in two distinct parts; the short anterior, muscular portion is about 0.2 mm. and usually, but not invariably, somewhat convoluted; the posterior portion is about 1.8 mm. long, occupying most of the body width where it opens into the simple and narrow intestine.

Paired, opposed genital organs occur in the female. In well developed specimens the anterior ovary rises just in front of the anterior branch of the uterus, extends to the posterior end of the oesophagus or slightly further, then curves back to join the uterus. The posterior ovary rises just behind its branch of the uterus, extends back to the anus, then returns to enter the uterus. The vulva is situated about two-thirds of the body length from the anterior end. From it the vagina runs directly posterior for about 0.2 mm. to receive the two branches of the uterus. An ovejector is present.

The male is recurved terminally. Two dissimilar spicules are present. The heavy right member, about 0.13 mm. long, is concave and equipped with backward pointing projections. The left one is simple and slender, about 0.5 mm. long, and bears a narrow wing rising in the last quarter of its length. Six conspicuous post-cloacal papillae and 12 pre-cloacal papillae are present. The last pre-cloacal members are not conspicuous. In addition to these a boss bearing a papilla-like structure is present just anterior to the cloaca. The single testis rises a short distance behind the oesophagus; it is simple and unconvoluted; no differentiation seems possible between it and the vas deferens. Neither caudal nor cephalic alae are apparent.

This was the only nematode found in *Catostomus commersonii*.

Conclusions

Though definitely limited in significance, it is interesting to note the interrelated host-parasite list. Lake trout were remarkably free from parasites, while the speckled trout were the most heavily parasitized hosts. The two other fish appear to be capable of maintaining infections of two of the parasites found in trout. *Ptychogonimus fontanus* is a strongly suckered trematode found in perch and speckled trout. The only acanthocephalid found was present in both suckers and the speckled trout.

Host Parasite List

Catostomus commersonii
- *Glaridacris intermedius* sp. nov.
- *Neoechinorhynchus cylindratus*
- *Rhabdochona laurentiana* sp. nov.

Cristivomer naymaycush
- *Eubothrium* (larval)
- Ichthyotaeniidae (larval)

Perca flavescens
- *Crepidostomum isostomum*
- *Ptychogonimus fontanus*

Salvelinus fontinalis
- *Crepidostomum cooperi*
- *Ptychogonimus fontanus*
- *Eubothrium salvelini*
- *Eubothrium* (larval)
- Ichthyotaeniidae (larval)
- *Neoechinorhynchus cylindratus*
- *Cystidicloides harwoodi*
- *Raphidascaris alius* sp. nov.

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Names of fish appearing here are those used by Hubbs (9).

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